

Long non-coding RNAs in motor neuron development and disease

Vamshidhar R. Vangoor  | Andreia Gomes-Duarte  | R. Jeroen Pasterkamp 

Department of Translational Neuroscience, University Medical Center Utrecht Brain Center, Utrecht University, Utrecht, The Netherlands

Correspondence

R. Jeroen Pasterkamp, Department of Translational Neuroscience, UMC Utrecht, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands.
Email: r.j.pasterkamp@umcutrecht.nl

Funding information

ALS Stichting Nederland; Dutch Epilepsiefonds, Grant/Award Number: WAR 18-05; Marie Skłodowska-Curie, Grant/Award Number: 721890

Abstract

Long non-coding RNAs (lncRNAs) are RNAs that exceed 200 nucleotides in length and that are not translated into proteins. Thousands of lncRNAs have been identified with functions in processes such as transcription and translation regulation, RNA processing, and RNA and protein sponging. lncRNAs show prominent expression in the nervous system and have been implicated in neural development, function and disease. Recent work has begun to report on the expression and roles of lncRNAs in motor neurons (MNs). The cell bodies of MNs are located in cortex, brainstem or spinal cord and their axons project into the brainstem, spinal cord or towards peripheral muscles, thereby controlling important functions such as movement, breathing and swallowing. Degeneration of MNs is a pathological hallmark of diseases such as amyotrophic lateral sclerosis and spinal muscular atrophy. lncRNAs influence several aspects of MN development and disruptions in these lncRNA-mediated effects are proposed to contribute to the pathogenic mechanisms underlying MN diseases (MNDs). Accumulating evidence suggests that lncRNAs may comprise valuable therapeutic targets for different MNDs. In this review, we discuss the role of lncRNAs (including circular RNAs [circRNAs]) in the development of MNs, discuss how lncRNAs may contribute to MNDs and provide directions for future research.

Abbreviations: 5-FU, 5-fluorouracil; AAV, adeno-associated virus; ABA, Allen brain atlas; AD, Alzheimer's disease; ADARB2, adenosine deaminase RNA-specific B2 (inactive); Ago2, Argonaute2; ALS, amyotrophic lateral sclerosis; arcRNAs, architectural RNAs; ASOs, antisense oligonucleotides; ATXN2, Ataxin-2; BDNF, brain-derived neurotrophic factor; bHLH, basic helix-loop-helix; bp, base pairs; C9-ALS, C9ORF72-associated ALS; C9ORF72, chromosome 9 ORF 72; ChIP, chromatin immunoprecipitation; circRNAs, circular RNAs; CLIP, cross-linking immunoprecipitation; CNS, central nervous system; CpG, cytosine-phosphorus-guanine; DBPs, DNA-binding proteins; *Dbr1*, debranching enzyme 1; DE, differential expression; DOX, doxorubicin; DPR, dipeptide repeat; dsRNA, double-stranded RNA; ER, endoplasmic reticulum; ESC, embryonic stem cells; FACS, fluorescence-activated cell sorting; fALS, familial ALS; FTD, frontotemporal dementia; FUS, fused in sarcoma; FUS/TLS, FUS/translocated in liposarcoma; GFP, green fluorescent protein; HCC, hepatocellular cancer; hESC, human embryonic stem cells; hiPSC, human-induced pluripotent stem cells; Hox, homeobox; hpf, hours post-fertilization; *hsp70*, heat-shock RNA ω ; HUVECs, human umbilical vein endothelial cells; IL, interleukins; INF, interferon; KCl, potassium chloride; LMC-MNs, limb innervating lateral motor column-motor neurons; lncRNAs, long non-coding RNAs; LPS, lipopolysaccharide; *Malat1*, metastasis-associated lung adenocarcinoma transcript 1; MATR3, Matrin 3; MEFs, mouse embryonic fibroblasts; *Meg3*, maternally expressed gene 3; mESC, mouse embryonic stem cells; *Miat*, myocardial infarction-associated transcript; miRNAs, MicroRNAs; MND(s), motor neuron disease(s); MN(s), motor neuron(s); MN-TFs, MN transcription factors; MNX1, Motor neuron and pancreas homeobox 1; NAC, nucleus accumbens; NAT, naturally occurring antisense transcript; NBs, nuclear bodies; ncRNA, non-coding RNA; *NEAT1*, nuclear-enriched abundant transcript 1; NONO, non-PoU domain-containing octamer-binding protein; *Olig2*, oligodendrocyte transcription factor 2; PAHs, polycyclic aromatic hydrocarbons; PBLCs, peripheral blood lymphocytes; PBMCs, peripheral blood mononuclear cells; PcG, polycomb group protein; PD, Parkinson's disease; piRNAs, Piwi-interacting RNAs; PLDs, prion-like domains; PRC1/2, polycomb repressive complex 1/2; PSPs, paraspeckle proteins; RA, retinoic acid; RAN, repeat-associated non-ATG-initiated translation; RBM14, RNA-binding motif protein 14; RBPs, RNA-binding proteins; *Rmst*, rhabdomyosarcoma 2-associated transcript; RNA-seq, RNA sequencing; rRNA, ribosomal RNA; sALS, sporadic ALS; *Sat III*, satellite III repeat RNA; SCA2, spinocerebellar ataxia type 2; SCZ, schizophrenia; SFPQ, splicing factor proline/glutamine-rich; SINES, short interspersed nuclear elements; siRNAs, small interfering RNAs; *Slc32a1*, solute carrier family 32 member 1; SMA, spinal muscular atrophy; SMN, spinal motor neuron; SN, substantia nigra; sncRNA, small non-coding RNA; SO, sense oligonucleotides; *SOD1*, superoxide dismutase1; SSOs, splice-switching oligonucleotides; TARDBP, TAR DNA-binding protein; TDP-43, TAR DNA-binding protein 43; TLR, toll-like receptors; UTR, untranslated region; VAPB, vesicle-associated membrane protein-associated protein B/C; Xist, X-inactive-specific transcript.

Vamshidhar R. Vangoor and Andreia Gomes-Duarte contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *Journal of Neurochemistry* published by John Wiley & Sons Ltd on behalf of International Society for Neurochemistry



KEYWORDS

long non-coding RNA, circular RNA, motor neuron development, spinal muscular atrophy, amyotrophic lateral sclerosis

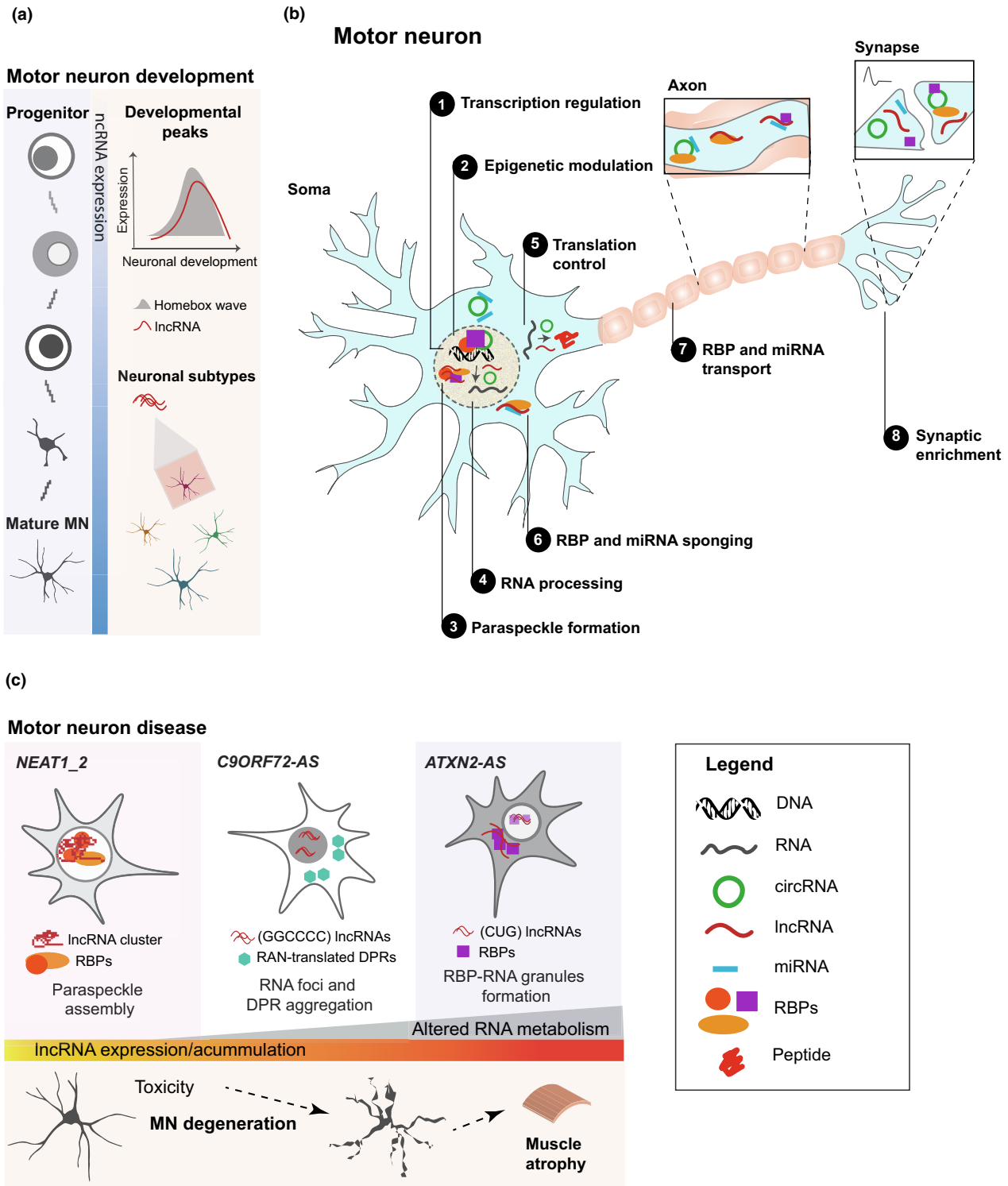
1 | INTRODUCTION

Over the past decade, the advent of high-throughput sequencing technologies coupled with advanced bioinformatics analysis has allowed an in-depth look into the non-coding part of the genome with exceptional resolution and scale. This has revolutionized our understanding of mammalian genome architecture, activity and regulation and led to the discovery that only $\pm 2\%$ of the human genome encodes proteins. This is remarkable given that the majority of nucleotides are transcribed, thus giving rise to an exquisitely high number of non-coding RNAs (ncRNAs) (Carninci et al., 2005; Djebali et al., 2012; Dunham et al., 2012). ncRNAs are subdivided on basis of their length into small ncRNAs (<200 nucleotides; e.g. microRNAs (miRNAs), small interfering RNAs (siRNAs) and piwi-interacting RNAs (piRNAs) and long ncRNAs (lncRNAs; >200 nucleotides), that differ in their biogenesis and function. lncRNAs show enormous functional diversity, influencing (post-)transcriptional and translational diversification of specific genes and gene networks (Qureshi & Mehler, 2012; Wang & Chang, 2011). It is estimated that approximately 2% of the human genome is transcribed into lncRNAs (Managadze et al., 2013; Palazzo & Lee, 2015). lncRNAs are mainly transcribed by RNA polymerase II (Guttman et al., 2009) and based on their location in the genome can be classified into the following five categories: (a) sense lncRNAs, transcripts transcribed from the sense strand of protein-coding genes and that contain one or more exons; (b) antisense lncRNAs, transcripts transcribed from the opposite strand of protein-coding genes and that contain one or more exons; (c) bidirectional lncRNAs, transcripts transcribed from the opposite strand that share the same promoter as another gene and that are usually located <1,000 base pairs (bp) away from each other in close genomic proximity; (d) intronic lncRNAs, transcripts

transcribed from intronic regions without showing overlap with exons; and (e) intergenic lncRNAs, independent transcripts located in between two genes (Derrien et al., 2012; Ma et al., 2013). Another interesting class of lncRNAs are circular RNAs (circRNAs). CircRNAs are produced by back-splicing events that result in a closed loop structure, where a covalent bond is formed by linking 5' (splice donor) and 3' (splice acceptor) RNA splice sites. CircRNAs can be transcribed from both exonic and intronic regions, but the majority of circRNAs are exonic (Chen, 2016; Jeck et al., 2013; Memczak et al., 2013).

Mechanistically, lncRNAs can act at the transcriptional and post-transcriptional level to affect transcription, RNA processing and translation (Elling et al., 2016). Mediation of chromatin interactions, involvement in the formation of nuclear structures (e.g. paraspeckles), formation of decoys for transcription factors or as scaffolds for DNA-binding proteins and RNA-binding proteins (RBPs), functioning as miRNA sponges and control of mRNA decay are core functions of lncRNAs (Figure 1; Marchese et al., 2017; Quinn & Chang, 2016). Even though the majority of lncRNAs is present in the nucleus, some lncRNAs are found in the cytoplasm (Kung et al., 2013; Noh et al., 2018; Rinn & Chang, 2012; Ulitsky & Bartel, 2013). In the cytoplasm, lncRNAs are involved in processes such as RNA editing, RNA splicing and protein machinery assembly for translation initiation (Noh et al., 2018; Rashid et al., 2016). The majority of lncRNAs are of low abundance. In general, expression of lncRNAs is approximately 10-fold lower as compared to protein-coding transcripts (Cabili et al., 2011; Ravasi et al., 2006). This difference can in part be explained by prominent differences in lncRNA expression between cell types and tissues, and the strong spatiotemporal regulation of lncRNA expression as compared to mRNA expression (Cabili et al., 2011; Field

FIGURE 1 Schematic illustration highlighting the roles of long non-coding RNAs (lncRNAs) in motor neuron (MN) development and disease. (a) Non-coding RNA (lncRNA and circRNA) expression is enhanced during MN differentiation suggesting roles in the regulation of MN development from progenitor stages to mature motor neurons (MNs). An interesting link has been established between lncRNA expression and homeobox (*Hox*) gene expression. lncRNAs influence *Hox* gene expression but can also be derived from the *Hox* gene cluster. A role for lncRNAs in the specification of neuron subtypes has been proposed. (b) lncRNAs and circular RNAs have been implicated in a wide range of cellular and molecular functions in developing MNs, all of which (in)directly impact on gene expression regulation. lncRNAs have been reported to play a role in transcription regulation, epigenetic modulation, paraspeckle formation, RNA processing, translational control, miRNA sponging and synaptic enrichment of RNAs and proteins. CircRNAs have been reported to play a role in transcription regulation, RNA processing, RBP and miRNA sponging, RBP and miRNA transport and synaptic enrichment of RNAs and proteins. (c) Several lncRNAs have been linked to MN disease, three examples of which are shown here. Nuclear-enriched abundant transcript 1 (*NEAT1*)₂ is a lncRNA that regulates paraspeckle assembly and that clusters with several RNA-binding proteins (RBPs) and inhibits their function. Changes in *NEAT1*₂ and paraspeckle assembly in general may contribute to the pathogenesis of amyotrophic lateral sclerosis (ALS). *C9ORF72-AS* is an antisense lncRNA that forms RNA foci and toxic dipeptide repeat (DPR) proteins in different cellular compartments because of the presence of hundreds to thousands hexanucleotide repeats in the 5' region of the *C9ORF72* locus in C9-ALS patients. In RNA foci *C9ORF72-AS* aberrantly interacts with RBPs which may contribute to cellular toxicity. *ATAXIN2-AS* is an antisense lncRNA that forms CUG repeat-containing RNA aggregates recruiting RBPs leading to neurotoxicity. In ALS, patients may carry an expanded CAG repeat region in the 5' coding part of the gene. Overall, lncRNA dysregulation may lead to altered RNA metabolism as a result of interference with RBPs and in case of C9-ALS the accumulation of toxic DPR proteins. These defects contribute to MN degeneration and muscle atrophy as observed in ALS patients



et al., 2019; Yan et al., 2013; Yunusov et al., 2016). Ubiquitously expressed lncRNAs are generally expressed at high levels, while cell type- or tissue-specific lncRNAs, such as those in MNs, are often expressed at lower levels (Jiang, Li., et al., 2016). In addition to relatively low levels of expression, many lncRNAs show low levels of evolutionary and sequence conservation (Basu et al., 2013; Ulitsky et al., 2011). It has been suggested that evolutionary conserved lncRNAs may have important general functions, while

species-specific lncRNAs could play relevant roles in biological processes that are crucial for a specific species (e.g. expansion of the brain in primates). Most of the lncRNAs discussed in this review are conserved among mammals (Table 1).

A remarkably large number of annotated lncRNAs, that is, approximately 40% (4,000 – 20,000 lncRNA genes), is expressed specifically in the brain (Derrien et al., 2012). In situ hybridization data from the Allen brain atlas show the expression of hundreds

Name	ncRNA	Reported species	References
<i>Meg3</i> , <i>Rian</i> and <i>Mirg</i>	lncRNA	All mammals	Ogata and Kagami, (2016) and Yen et al., (2018)
<i>CAT7</i>	lncRNA	All mammals	Ray et al., (2016)
<i>Hoxb5os</i>	lncRNA	Mouse and human	Papaioannou et al., (2019)
<i>Gm12688/</i> <i>Gm14204</i>	lncRNA	Mouse, human (NA)	Rizvi et al., (2017)
<i>LncMN-1,-2,-3</i> and <i>Lhx1os</i>	lncRNA	Mouse and human	Biscarini et al., (2018)
<i>Lncrps25</i>	lncRNA	<i>Danio rerio</i> and human	Gao et al., (2020) and Ulitsky et al., (2011)
<i>Malat1</i>	lncRNA	All mammals	Ulitsky et al., (2011)
<i>Rmst</i>	lncRNA	Birds and mammals	Chodroff et al., (2010)
<i>Xist</i>	lncRNA	All mammals	Johansson et al., (2014)
<i>Miat (Gomafu)</i>	lncRNA	All mammals	Chodroff et al., (2010); Sone et al., (2007)
<i>FUS-linked circRNAs</i>	circRNA	Mouse and human	Errichelli et al., (2017)
<i>SMN circRNAs</i>	circRNA	Mouse and human	Ottesen et al., (2019)
<i>NEAT1</i>	lncRNA	Mouse and human	Clemson et al., (2009)
<i>BDNFOS</i>	lncRNA	Primates	Lipovich et al., (2012)
<i>TFEBα</i>	lncRNA	Human	Davis et al., (2003)
<i>Myolinc</i>	lncRNA	Mouse	Militello et al., (2018)
<i>SATIII</i>	lncRNA	<i>Drosophila melanogaster</i> and human	Chung et al., (2018)

Note: Overview of the reported conservation of lncRNAs discussed in this review. lncRNAs are listed according to their discussion in the main text.

Abbreviations: FUS, fused in sarcoma; Meg3, maternally expressed gene 3; NA, not annotated; SMN, survival motor neuron.

of lncRNAs in specific regions of the mouse brain, in specific types of neural cells and even in specific subcellular compartments. Several of these expression patterns are similar to those described for coding mRNAs, hinting at important neuronal functions for lncRNAs (Mercer et al., 2008). Indeed, lncRNAs have been linked to processes such as neuron development, neurite growth, synaptic transmission, memory consolidation and ageing (Derrien et al., 2012; Mehler & Mattick, 2007; Mercer et al., 2008; Pereira Fernandes et al., 2018; Shi et al., 2017). In this review, we specifically focus on the proposed role of lncRNAs in motor neurons (MNs). MNs are a group of neurons that have their cell bodies in the cortex (upper MNs) or in the brainstem and spinal cord (lower MNs) and project axons into the brainstem, spinal cord or towards peripheral muscles. These projections control essential functions such as movement, breathing and swallowing. Not surprising given their important functions, selective degeneration of MNs is a hallmark of motor neuron diseases (MNDs) such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). Here we review the role of lncRNAs in the development and functions of MNs, discuss how lncRNAs may contribute to MNDs and provide directions for future research.

TABLE 1 Evolutionary conservation of motor neuron lncRNAs

2 | LNCRNAs IN MOTOR NEURON DEVELOPMENT

2.1 | Linear lncRNAs in developing motor neurons

MN development is complex and depends on the interplay between different molecular factors. Several lines of experimental evidence link lncRNAs to the development of MNs (Figure 1a,b, see also Table 2).

2.2 | *Meg3*

The imprinted mammalian *Dlk1-Dio3* locus produces multiple lncRNAs from the maternally inherited allele. Profiling of differentiating embryonic stem cell (ESC)-derived MNs revealed a predominant and gradual enrichment of maternally expressed gene 3 (*Meg3*) and other lncRNAs from the imprinted *Dlk1-Dio3* locus (*Rian* and *Mirg*) at post-mitotic stages of rostral MN development (Y. P. Yen et al., 2018). This enrichment was found not only in vitro in ESC-MNs but also in vivo in mouse spinal cord. Interestingly, both the over-expression of MN transcription factors and the treatment with retinoic acid



TABLE 2 Overview of the expression and proposed function of lncRNAs in motor neuron development

Name	ncRNA	Regulation	Observation	Mechanism	References
Meg3	lncRNA	Up-regulated; spatial regulation	Regulated throughout embryonic stem cells–motor neuron (ESC-MN) differentiation; enriched in the nucleus	Epigenetic regulation of <i>Hoxa4:Hoxc5</i> expression	Yen et al., (2018)
CAT7	lncRNA	Up-regulated	Regulated during early stages of human ESC-MN differentiation	Regulation of polycomb repressive complex 1 (PRC1) associated genes	Ray et al., (2016)
<i>Hoxb5os</i>	lncRNA	Up-regulated	Regulated throughout ESC-MN differentiation	Tbd	Rizvi et al., (2017)
<i>Gm12688/Gm14204</i>	lncRNA	Cell type-specific expression	Uniquely expressed in V1/V1 and V2b GABAergic interneurons	Tbd	Rizvi et al., (2017)
<i>LncMN-1,-2,-3</i> and <i>Lhx1os</i>	lncRNA	Cell type-specific expression; up-regulated	Specifically enriched in MNs; regulated during differentiation of mouse ESC (mESC)/ human-induced pluripotent stem cells (hiPSC)-derived MNs	Tbd	Biscarini et al., (2018)
<i>Lncrps25</i>	lncRNA	Down-regulated	Knockdown reduces swimming activity because of defects in primary MNs	Via <i>olig2</i> (Tbd)	Gao et al., (2020)
<i>Malat1, Meg3, Rmst, Xist</i> and <i>Miat</i>	lncRNA	Spatial distribution	Specifically enriched in somatodendritic/axonal fractions	Tbd	Briese et al., (2016)
<i>c-1, c-2, c-13, c-16, c-48, c-80, c-82, c-84, c-88</i>	circRNA	Up-regulated	Regulated during mESC/hiPSC-derived MN differentiation	Tbd	Errichelli et al., (2017)
<i>Human circSMN</i>	circRNA	Multiple isoforms produced	Primate specificity of SMN-derived circRNAs	Tbd	Ottesen et al., (2019)

Abbreviations: hiPSC, human-induced pluripotent stem cells; Meg3, maternally expressed gene 3; mESC, mouse embryonic stem cells; Tbd, to be determined.

in a normally maternally inherited methylated region context (IG-DMR^{matΔ} ESC) induced *Meg3* expression. Therefore, these factors, that are crucial for MN development, may drive *Meg3* expression during differentiation of ESCs into rostral brachial MNs. *Meg3* was found to be enriched in the nucleus of MNs while being chromatin-associated. This strongly suggested a role in gene regulation. Indeed, by combining gene knockdown and chromatin immunoprecipitation, *Meg3* was shown to facilitate polycomb repressive complex 2 (PRC2)-Jard2 interactions in post-mitotic MNs. *Meg3* knockdown led to a global down-regulation of H3K27me3 and rostral Homeobox (*Hox*) genes, and an up-regulation of MN progenitor genes (*Pax6* and *Irx3*) and of caudal *Hox* genes (a *Hox5* to *Hoxc8* shift). *Hox* genes are transcription factors that regulate MN fate along the rostro-caudal axis of the central nervous system (CNS). Based on these and other findings it was proposed that *Meg3* facilitates PRC2:Jard2 complex formation to perpetuate rostral MN cell fate by maintaining the silenced epigenetic state of MN progenitor and caudal *Hox* genes (Y. P. Yen et al., 2018).

2.3 | CAT7

Another screen designed to detect lncRNAs co-precipitating with polycomb repressive complex 1 (PRC1) from chromatin identified multiple lncRNAs that influence the expression of polycomb group protein-regulated genes. Loss of a novel lncRNA, CAT7, led to an increase in MN and pancreas homeobox 1 (MNX1 or HB9) expression in human embryonic stem cells-derived MNs (Ray et al., 2016). MNX1 is a transcription factor that regulates the formation of pancreatic beta islets and MNs (Bonfond et al., 2013; Van Arensbergen et al., 2010). During MN development, MNX1 expression is silenced at early stages of differentiation as a result of PRC1 binding to the MNX1 locus (Van Arensbergen et al., 2010). CAT7 enhances the binding of PRC1 to the MNX1 locus before activation of pathways that determine MN fate. CAT7 shares high sequence similarity to a non-syntenic *Danio rerio* (zebrafish) analog, *zcat7l*. Depletion of *zcat7l* by morpholino interference resulted in microcephaly at 48 hr post-fertilization (hpf) and mortality starting on day 5. These phenotypes were successfully reverted by co-expression of the human CAT7 lncRNA isoform, indicating possible conserved functions between these non-syntenic lncRNAs. The phenotypes found following *zcat7l* depletion were also observed following knockdown of the zebrafish polycomb complex proteins *bmi1a/b*, and *zcat7l* and *bmi1a/b* genetically interacted in combined knockdown experiments. Overall, these data indicate a functional link between CAT7/*zcat7l* lncRNAs and PRC1 in human and zebrafish. More generally, these findings show that lncRNAs can regulate and promote transcription activation at specific loci (Ray et al., 2016).

2.4 | Gm12688 and Gm14204

In another study, single-cell topological RNA sequencing analysis was used to characterize the mechanisms underlying in vitro

differentiation of mouse embryonic stem cell (mESC)-derived MN cultures (Rizvi et al., 2017). Specific temporal regulation of lncRNAs was found during pluripotency, at the neural progenitor stage and during the transition to neuronal maturation. Different stages of MN differentiation are characterized by waves of *Hox* gene activation (Figure 1a). Intriguingly, these waves were accompanied by an up-regulation of lncRNAs that originate from the opposite strand of these genes. This co-expression is in line with previous reports identifying lncRNAs as regulators of *Hox* gene clusters (Dinger et al., 2008; Ponjavic et al., 2009; Rinn et al., 2007; Wang et al., 2011). In addition, several lncRNAs were detected in other neuron types in the cultures, which contain MNs and concomitant cell types (i.e. those found in the spinal cord). For example, *Gm12688*, an intergenic lncRNA located near *Foxd3* and transcribed from the opposite strand, was found in V1 interneurons. *Gm14204*, an intergenic lncRNA located near *Solute carrier family 32 member 1 (Slc32a1)* and transcribed from the opposite strand, was restricted to V1 and V2b GABAergic interneurons (Rizvi et al., 2017). The discovery of these intergenic lncRNAs suggests that lncRNAs may play a role in neuronal diversification in the spinal cord.

2.5 | Lhx1os, Lnc-MN1, LncMN-2 and LncMN-3

Stage-specific expression of lncRNAs was also detected in an analysis of the long non-coding transcriptome of mESC- and human-induced pluripotent stem cells (hiPSC)-derived MNs purified by fluorescence-activated cell sorting (FACS). The expression of several lncRNAs was restricted to MNs, as compared to absence of signal in green fluorescent protein-negative cells (i.e. MN progenitors and interneurons). Interestingly, these lncRNAs showed a predominant cytoplasmic expression (Biscarini et al., 2018). Expression of a subset of mouse lncRNAs (*Lhx1os*, *2610316D01Rik*, *5330434G04Rik* and *A730046J19Rik*) was specifically increased at later stages of ESC-MN development (from day 4 onwards), during which MN progenitors develop into post-mitotic MNs and differentiate further. This lncRNA cluster was conserved in human (renamed *Lhx1os*, *Lnc-MN1*, *LncMN-2* and *LncMN-3*) and showed a similar mode of expression regulation during hiPSC-derived MN development. These results further confirm the role of lncRNAs in MN differentiation and hint at strong evolutionary conservation of the mechanism-of-action and function of lncRNAs in MNs.

2.6 | Malat1, Meg3, Rmst, Xist and Miat

In addition to studies reporting general changes in lncRNA expression, other work has focused on the spatial enrichment of these non-coding RNAs in specific subcellular compartments. Initially, lncRNAs had predominantly been detected in the nucleus and were therefore studied for potential roles in the regulation of gene expression (Sun et al., 2018; Ulitsky & Bartel, 2013). The presence of lncRNAs in other subcellular compartments hinted at the exciting possibility



of additional and different molecular functions. Briese et al. (2016) investigated the abundance of several lncRNAs in the axonal or somatodendritic compartment, including that of metastasis-associated lung adenocarcinoma transcript 1 (*Malat1*), *Meg3*, rhabdomyosarcoma 2-associated transcript (*Rmst*), X-inactive-specific transcript (*Xist*) and Myocardial infarction-associated transcript (*Miat*). While some lncRNAs showed similar levels in different compartments, *Meg3* and *Rmst* were enriched in somatodendritic and axonal fractions, respectively (Briese et al., 2016). The molecular mechanisms dictating this highly specific distribution are unknown. However, a number of lncRNAs can interact with proteins and it is plausible that protein:RNA complexes play a role in compartment-specific lncRNA distribution (Briese et al., 2016; Quan et al., 2017).

2.7 | *Lncrps25*

While many of these studies reveal interesting lncRNA expression patterns that hint at roles during MN differentiation, recent work has also begun to explore the functional role of lncRNAs in MNs in vivo. For example, the lncRNA *Lncrps25* was found to be strongly expressed in the embryonic zebrafish CNS and co-expressed with *mxn1*, while *Lncrps25* knockdown induced body abnormalities and striking changes in locomotor behaviour, that is, decreased swimming distance and speed (Gao et al., 2020). These phenotypes were caused by defects in primary MNs that showed reduced axon length and branching. *Lncrps25* depletion led to a reduced expression of oligodendrocyte transcription factor 2 (*olig2*) in brain and spinal cord. *Olig2* co-expression could partially rescue the motor phenotypes caused by *Lncrps25* depletion. *Olig2* is part of the *Olig* gene family that encode for basic helix-loop-helix transcription factors that are required for MN and oligodendrocyte development (Lu et al., 2002; Park et al., 2002). Based on the effect of *Lncrps25* depletion, and *Lncrps25* and *olig2* expression patterns, it was proposed that *Lncrps25:olig2* interactions occur at the neural plate stage to eventually affect MN development (Gao et al., 2020). However, the precise mechanistic details of this lncRNA-mRNA pathway remain to be defined. A recent study focusing on defining lncRNA networks in oligodendrocytes identified the oligodendrocyte-specific lncRNA *LncOL1*. *LncOL1* is involved in oligodendrocyte differentiation and CNS (re)myelination (He et al., 2017). Interestingly, a peak in genomic occupancy by *Olig2* was detected around the transcription start site of *LncOL1*, suggesting a role for *Olig2* in the transcriptional regulation of *LncOL1*. Therefore, *Olig2* appears to be a shared factor in the regulation of MN and oligodendrocyte differentiation through interactions with different lncRNAs (*Lncrps25* and *LncOL1*). This is intriguing since the specification of MN and oligodendrocytes from ventral progenitor cells is a sequential process mediated by *olig2* (Ravanelli & Appel, 2015). Further, oligodendrocytes are known to play a role in MNDs such as ALS. Oligodendrocyte dysfunction contributes to MN excitability and death, and consequently to subsequent oligodendrocyte-related pathology (Ferraiuolo et al., 2016). It will be interesting to determine how *Olig2:lncRNA* networks interact during

MN and oligodendrocyte development, and how their dysfunction may lead to disease.

2.8 | CircRNAs in developing motor neurons

CircRNAs are highly enriched in the brain, dynamically regulated and conserved across species (Rybak-Wolf et al., 2015). The mechanisms that cause brain-enriched circRNA expression remain to be determined. One explanation is that many circRNAs derive from genes that are enriched in brain tissue, but the existence of neuron-specific biogenesis mechanisms has also been proposed (van Rossum et al., 2016). Another interesting observation is that whereas linear lncRNAs often display cell type-specific expression (e.g. in MNs), cell type-specific circRNAs are rare. This difference may arise from the fact that whereas many lncRNAs originate from intergenic stretches of the genome that lack protein-coding genes, circRNAs are generally produced from protein-coding transcripts (Memczak et al., 2013; Ransohoff et al., 2018; Salzman et al., 2012). MN-specific circRNAs therefore mainly arise from MN-specific genes, while the intergenic regions that produce other lncRNAs may be susceptible to different kinds of (post)transcriptional regulation. CircRNAs are differentially expressed across brain regions, subcellular compartments and regulated at pre- and postnatal stages (Chen & Schuman, 2016; Memczak et al., 2013; Rybak-Wolf et al., 2015; Venø et al., 2015; You et al., 2015). Many of these RNA molecules derive from genes that encode for synaptic proteins or proteins with essential roles in neural development. Further, the expression of several circRNAs is influenced by neuronal activity and they are shown to accumulate during ageing (Venø et al., 2015; Westholm et al., 2014). Together, these observations hint at roles for circRNAs in a wide range of neuronal processes in brain development, function and disease (van Rossum et al., 2016).

The functional roles and mechanism-of-action of most circRNAs remain poorly understood. A detailed discussion of the function of circRNAs is beyond the scope of this review, but several other recent reviews cover our current knowledge of the molecular effects and functional roles of circRNAs (Ebbesen et al., 2016; Meng et al., 2019; van Rossum et al., 2016; Salzman, 2016; Sekar & Liang, 2019). Translation into small peptides, miRNA sponging and sequestration of RBPs are some of the currently accepted mechanisms by which circRNAs can act (Li et al., 2018).

2.9 | FUS-linked circRNAs

CircRNAs have also been detected in MNs. Hundreds of circRNAs were found in mESC-derived MNs and, similar to that has been demonstrated for other lncRNAs, their expression increased during MN differentiation (Errichelli et al., 2017). The majority of identified circRNAs was conserved in hiPSC-derived MNs. In addition to expression profiling, this study also provided insight into the mechanisms underlying circRNA biogenesis in MNs. The

ALS-associated protein fused in sarcoma (FUS) was shown to promote circRNA formation in MNs without affecting their linear counterparts. FUS is an RBP with roles in RNA splicing and may contribute to circRNA formation by regulating the back-splicing events that lead to RNA circularization. In line with this idea, cross-linking immunoprecipitation (CLIP) assays revealed a specific enrichment of FUS at circularizing exon–intron regions of selected circRNAs (c-1, c-2, c-13, c-16, c-48, c-80, c-82, c-84, c-88) (Errichelli et al., 2017; Verheijen & Pasterkamp, 2017).

2.10 | SMN circRNAs

Although the biogenesis and functions of circRNAs are often evolutionarily conserved, in some instances the abundance, the presence of specific isoforms or functions of circRNAs are species-specific (Rybak-Wolf et al., 2015). Here, it is worth mentioning the human *survival motor neuron* (SMN) locus, which is able to originate a surprisingly large number of different exonic circRNAs (Ottesen et al., 2019). SMN protein is essential for MN development and survival and its loss causes SMA (Chaytow et al., 2018; Fallini et al., 2012; Lefebvre et al., 1995). Most of the reported functions of SMN are linked to RNA processing. However, it is known that SMN protein accumulates in the axons of MNs during development hinting at roles in, for example, local gene expression regulation (Gabanella et al., 2005; Giavazzi et al., 2006; Groen et al., 2013; Zhang et al., 2006). In addition to SMN protein, the SMN locus also generates a vast array of circRNAs. Using intron–exon site complementarity analysis in MN-like NSC-34 cells (mouse MN-like hybrid cell line), the mouse *Smn* locus was predicted to give rise to a considerably lower number of circRNAs as compared to the human SMN locus (Ottesen et al., 2019). These differences seem to result from a lower number of short interspersed nuclear elements in the intronic regions of the mouse *Smn* gene. A substantial number of SMN-derived circRNAs was specific to primates. It is therefore possible that specific circRNAs may be directly linked to MN functions that have become more important during evolution. The consequence of the species-specific expression of SMN-derived circRNAs and the functional roles of these lncRNAs remain to be determined. Nevertheless, this insight highlights the need for more knowledge on how genetic differences influence non-coding RNA biogenesis and function.

3 | LNCRNAs IN MOTOR NEURON DISEASE

lncRNAs are strongly regulated by exposure to drugs, environmental factors (e.g. toxins) or as a consequence of pathological situations. In many instances, regulation of lncRNA expression confers neuroprotection or induces anti-apoptotic responses (Table 3). Given this regulation of lncRNAs, their dynamic expression patterns in MNs and their emerging roles in MN development and function, it is not

surprising that lncRNA dysregulation has been implicated in MNDs. Understanding the mechanisms-of-action and functions of lncRNAs may assist the development of new therapies for MNDs. Here, we discuss and summarize the expression and functional role of lncRNAs in different MN-related diseases (see also Table 4), extending on previous reviews (Chen & Chen, 2020; Gagliardi, et al., 2018).

3.1 | Amyotrophic lateral sclerosis (ALS)

ALS is a fatal disease characterized by the progressive loss of MNs in brain and spinal cord. About 90%–95% of cases lack a clearly identifiable hereditary or environmental cause classically being referred to as sporadic ALS (sALS). About 5%–10% of cases are defined as familial ALS (fALS) because of demonstration of the direct inheritance (Renton et al., 2014), although the simple distinction between sALS and fALS is no longer tenable (Al-Chalabi et al., 2016). In most countries, the prevalence of ALS is approximately four to six cases per 100,000 people, with a median age of onset of 65 years for sALS, whereas this is 10 years earlier for fALS cases (Al-Chalabi & Hardiman, 2013; van Es et al., 2017). As disease progresses, a large proportion of upper (corticospinal) MNs, projecting from the motor cortex to the brainstem and spinal cord, and bulbar and spinal MNs, projecting to skeletal muscles, degenerate. This ultimately leads to spasticity and muscle atrophy which eventually results in weakness and paralysis. Eventually, patients die as a result of paralysis of respiratory muscles within 3–5 years after symptom onset (van Es et al., 2017).

The causative mechanisms that lead to MN degeneration and ALS remain incompletely understood. Mutations in more than 30 genes have been linked to ALS, and based on the functions of these genes, multiple disease pathways have been proposed and investigated. The most commonly mutated genes are *chromosome 9 ORF 72* (*C9ORF72*; hexanucleotide (GGGGCC)_n (G₄C₂) repeat expansion in a non-coding genomic region), superoxide dismutase1, TAR DNA-binding protein and *FUS* (Renton et al., 2014). These genes regulate molecular pathways controlling key cellular events such as RNA biology, protein turnover and axonal transport (Burk & Pasterkamp, 2019; Robberecht & Philips, 2013).

The RBPs TAR DNA-binding protein 43 (TDP-43) and *FUS* are normally located in the nucleus and influence RNA metabolism. In ALS MNs, an abnormal accumulation of these proteins is observed in the cytoplasm that is thought to contribute to MN degeneration because of effects on RNA processing and other RNA-related mechanisms (Blokhuys et al., 2013). Further, the ALS-associated hexanucleotide repeat expansion in the *C9ORF72* locus causes mutant *C9ORF72* pre-mRNA to accumulate in RNA foci leading to accumulation of RBPs (Gendron et al., 2013; Renton et al., 2014). Given these and other data supporting a prominent role for defects in RNA biology in ALS, it is not surprising that lncRNAs also contribute to the development of ALS and other MNDs. Here we discuss a few lncRNAs that have been implicated in the pathogenesis of ALS or SMA.

TABLE 3 Expression changes in motor neuron lncRNAs in response to exposure to external factors or drugs

LncRNA	Treatment/stimulus	Response	(Neuro) protection or Disease onset	System	References
<i>Meg3</i>	5-aza-2-deoxycytidine (demethylating agent)	Up-regulation	Tbd	Human breast cancer tumour cell lines; Human meningioma cell line; human hepatocellular cancer (HCC) cell lines	Braconi et al., (2011); Zhang et al., (2010) and Zhao et al., (2005)
	Hypoxia	Up-regulation	Tbd	Human umbilical vein endothelial cells (HUVECs)	Michalik et al., (2014)
	Serum starvation	Up-regulation	Tbd	Mouse embryonic fibroblasts (MEFs)	Wang, Liang, et al. (2019)
	Heroin	Up-regulation	Tbd	Human nucleus accumbens (NAc)	Michelhaugh et al., (2011)
<i>Hoxb5os</i>	Serum starvation	Down-regulation	Tbd	MEFs	Wang, Liang, et al. (2019)
<i>Malat1</i>	Polycyclic aromatic hydrocarbons (PAHs) exposure	Up-regulation	Tbd	Peripheral blood lymphocytes (PBLs) of male coke oven workers	Gao et al., (2016)
	Hypoxia	Up-regulation	Tbd	HUVECs; Several tissues of mice exposed to hypoxia (including brain)	Lelli et al., (2015); Michalik et al., (2014)
	X-irradiation	Up-regulation	Tbd	Human lymphoblast cell line TK6	Chaudhry, (2014)
	β -Asarone	Down-regulation	Neuroprotective	In vitro and in vivo (mice)	Zhang et al., (2016)
	doxorubicin (DOX), 5-fluorouracil (5-FU), Mitomycin	Up-regulated	Anti-apoptotic	DOX, 5-FU and Mitomycin-resistant HCC cells	Yuan et al., (2016) and Zhang et al., (2020)
	Heroin	Up-regulation	Tbd	Human NAc	Michelhaugh et al., (2011)
<i>Rmst</i>	X-irradiation	Up-regulation	Tbd	Human lymphoblast cell line TK6	Chaudhry, (2014)
<i>Xist</i>	DOX	Up-regulation	Tbd	DOX-resistant colorectal cancer tissues and cells	Zhu et al., (2018)
<i>Miat (Gomafu)</i>	Cisplatin (CDDP)	Up-regulation	Tbd	(CDDP)-resistant H1299 cells	Wu et al., (2020)
	Potassium chloride (KCl)	Down-regulation	Might contribute to alternative splicing observed in schizophrenia (SCZ)	Neuronal stimulation of mouse primary cortical neurons and iPSC-derived neurons. Reduced expression in SCZ patient tissue	Barry et al., (2014)
	Serum starvation	Down-regulation	Tbd	MEFs	Wang, Liang, et al. (2019)
	Heroin and Cocaine	Up-regulation	Tbd	Human NAc	Albertson et al., (2006) and Michelhaugh et al., (2011)
<i>Neat1</i>	Hypoxia	Up-regulation	Tbd	Several tissues of mice exposed to hypoxia (including brain). In vitro and in vivo (mice)	Lelli et al., (2015)
	Serum starvation	Up-regulation	Tbd	MEFs	Wang, Liang, et al. (2019)
	LPS	Up-regulation	KD improves protection against LPS-induced myocardial injury.	In vivo (mice)	Wang, Liu, et al. (2019)

(Continues)



TABLE 3 (Continued)

LncRNA	Treatment/stimulus	Response	(Neuro) protection or Disease onset	System	References
	H ₂ O ₂ induced (oxidative stress)	Up-regulation	Increased viability. May contribute to neuroprotection in Huntington's disease	N2A cells	Sunwoo et al., (2017)
	Heroin	Up-regulation	Tbd	Human NAc	Michelhaugh et al., (2011)
	KCl	Up-regulation	Tbd	SH-SY5Y cells	Lipovich et al., (2012)
	Fenofibrate and Simvastatin	Up-regulation	Neuroprotective	SH-SY5Y cells	Simchovitz et al., (2019)
BDNFOS	KCl depolarization	Down-regulation	Negative regulator of brain-derived neurotrophic factor (BDNF)	SH-SY5Y cells	Lipovich et al., (2012)

Note: Overview of the expression changes in lncRNAs in response to various stimuli and their proposed effects that are discussed in this review.

Abbreviations: HCC; hepatocellular cancer; hiPSC; human-induced pluripotent stem cells; LPS; lipopolysaccharide; *Malat1*; metastasis-associated lung adenocarcinoma transcript 1; MEFs, mouse embryonic fibroblasts; Meg3, maternally expressed gene 3; Tbd, to be determined.

3.2 | NEAT1

NEAT1 is a well-characterized lncRNA that organizes nuclear structures called 'paraspeckles'. Paraspeckles contain proteins involved in transcription and RNA processing (Clemson et al., 2009; Fox & Lamond, 2010). So far, two isoforms of *NEAT1*, *NEAT1_1* and *NEAT1_2*, have been described that are spliced by alternative processing of the 3'-untranslated region (Fox et al., 2018; Naganuma et al., 2012; Yamazaki et al., 2018). *NEAT1* is classified as one of the architectural RNAs (arcRNA), that function by providing essential scaffolds for the formation of nuclear bodies, with *NEAT1_2* playing a predominant role in paraspeckle formation (Chujo et al., 2016; Naganuma et al., 2012). Paraspeckle formation is a tightly regulated process that occurs parallel to *RNApolIII* transcription of *NEAT1_2* and binding of several paraspeckle proteins (PSPs) to *NEAT1* (Mao et al., 2011; Yamazaki et al., 2018). So far, more than 60 PSPs have been identified, most of which are RBPs (Yamazaki & Hirose, 2015). RNAi studies identified seven essential PSPs that are able to form a paraspeckle with ~ 50 *NEAT1_2* molecules (Chujo et al., 2017; Naganuma et al., 2012). Six of the seven essential PSPs possess prion-like-domains, of which FUS and RNA-binding motif protein 14 are shown to be required to form paraspeckles in vivo (Hennig et al., 2015; Yamazaki & Hirose, 2015). Functionally, PSPs interact with specific domains of *NEAT1* in the formation of paraspeckles. These domains were elucidated by systemic deletion of parts of *NEAT1_2* using CRISPR/Cas9-mediated genome editing, revealing domains important for: 1) RNA stabilization of *NEAT1_2* (C-terminal domain and N-terminal TH structure), 2) paraspeckle assembly (middle domain) and 3) isoform switching (polyadenylation signal containing domain; Yamazaki et al., 2018). The middle domain of *NEAT1_2* further contains redundant functional subdomains responsible for paraspeckle assembly. By performing CLIP-seq it was confirmed that PSPs non-PoU domain-containing octamer-binding protein (NONO), splicing factor proline/glutamine-rich (SFPQ) and FUS bind several of the *NEAT1_2* subdomains. Paraspeckle assembly could be rescued by artificial tethering of a *NEAT1_2* mutant lacking functional subdomains with one of the PSPs (NONO, SFPQ or FUS). The phase-separation property of paraspeckles was shown by treatment with 1,6-hexanediol, which not only disrupted paraspeckle structure but also disturbed interactions between NONO and RBPs that contain prion-like-domains like FUS. It is speculated that FUS through its PLD recruits several PSPs, leading to the formation of FUS oligomers thereby mediating paraspeckle assembly via phase separation (Yamazaki et al., 2018). Overall, these studies highlight the key role of *NEAT1_2* subdomains in paraspeckle assembly through phase separation.

Several lines of experimental evidence suggest that the size and number of paraspeckles is influenced by the expression level of *NEAT1*. Physiologically, *NEAT1* and paraspeckles are not detectable in human embryonic stem cells, but differentiation leads to the induction of *NEAT1* expression and formation of paraspeckles. Knockdown of *NEAT1* in HeLa cells leads to the loss of paraspeckles and increased nucleocytoplasmic export of mRNAs bearing

**TABLE 4** Overview of the expression and proposed functions of lncRNAs in motor neuron disease

Name	ncRNA	Disease	Regulation	Function	References
NEAT1	lncRNA	ALS	Up-regulated at early stage	Regulates paraspeckle formation, increased NEAT1 expression leads to neurotoxicity	Clemson et al., (2009); Nishimoto et al., (2013) and Suzuki et al., (2019)
C9ORF72-AS	antisense RNA	ALS	Up-regulated	Forms RNA foci that recruit RBPs, DPR protein formation via repeat-associated non-ATG-initiated (RAN) translation leading to neurotoxicity	Cheng et al., (2019); Mizielinska et al., (2014); Mori, et al. (2013); Sareen et al., (2013); Swinnen et al., (2018) and Wen et al., (2014)
ATXN2-AS	antisense RNA	ALS	Up-regulated	Repeat expansion RNA induces neurotoxicity	Li, Sun, et al. (2016)
SMN-AS	antisense RNA	SMA	Up-regulated	Recruits polycomb repressive complex 2 (PRC2) complex to the SMN gene to suppress SMN expression	d'Ydewalle et al., (2017) and Woo et al., (2017)
ZEB1-AS, ZBTB11-AS	antisense RNA	ALS	Up-regulated in blood samples (peripheral blood mononuclear cells [PBMCs])	Tbd	Gagliardi, et al. (2018)
UBXN7-AS, ATG10-AS, ADORA2A-AS	antisense RNA	ALS	Up-regulated in blood samples (PBMCs)	Tbd	Gagliardi, et al. (2018)
hsa_circ_0001173, hsa_circ_0043138, hsa_circ_0088036	circRNA	ALS	Up-regulated in blood samples (PBMCs)	Biomarker potential	Dolinar et al., (2019)

Abbreviations: ATXN2, Ataxin-2; NEAT1, nuclear-enriched abundant transcript 1; SMN, survival motor neuron; Tbd, to be determined.

Alu repeats, implicating *NEAT1* function in mRNA export (Chen & Carmichael, 2009). Induction of *NEAT1* levels concomitant with paraspeckle enlargement has been observed under different conditions, for example, during viral infection and myotube differentiation (Sunwoo et al., 2009; Zhang et al., 2013). Further, proteasome inhibition in HeLa cells by MG132 led to strong up-regulation of *NEAT1* together with the elongation of paraspeckles and sequestration of the transcription factor SFPQ by *NEAT1*. In contrast, *NEAT1* knockout mouse embryonic fibroblasts display the loss of paraspeckles and are more susceptible to proteasome inhibitor-mediated cell death, suggesting a pro-survival role for paraspeckles under stressful conditions (Hirose et al., 2014).

As indicated above, *NEAT1* interacts with several RBPs involved in ALS (i.e. FUS and TDP-43), both in human brain tissue and in cultured cells (Nishimoto et al., 2013; Tollervey et al., 2011). In early onset ALS patient tissue, not only the frequency of paraspeckle formation is highly increased but also the co-localization of *NEAT1* with TDP-43 and FUS in paraspeckles is enhanced. Further, by performing interaction and localization studies it was concluded that *NEAT1* acts as a scaffold for these RBPs in the nuclei of ALS MNs (Nishimoto et al., 2013). Subsequent work confirmed these observations, showing an increase in paraspeckle formation in the spinal cords of sALS and fALS patients (Shelkovichnikova et al., 2018). ALS-associated RBPs not only interact with *NEAT1* but also regulate *NEAT1* expression. Deletion of FUS, TDP-43 or Matrin 3 (MATR3) leads to increased *NEAT1_2* RNA levels (Banerjee et al., 2017; Naganuma et al., 2012). Paraspeckles are known to exert anti-apoptotic activity and can increase cell viability under stressful conditions (Hirose et al., 2014; Shelkovichnikova et al., 2018). Their increase at early disease stages in ALS could hint at compensatory mechanisms to increase MN survival. Mutations in FUS lead to impaired paraspeckle formation by dysregulating *NEAT1* transcription and mis-assembly of other paraspeckle proteins that are required during adequate stress responses (Shelkovichnikova et al., 2014). This disrupts neuroprotective mechanisms and may contribute to the aggressive disease phenotype (early onset and fast progression) observed in FUS-ALS cases (An et al., 2019). Although these studies suggest that ALS-related RBP mutations affect *NEAT1* expression and may thereby influence paraspeckle formation, the precise role of *NEAT1* in ALS pathogenesis is incompletely understood. A recent study shows that the activation and up-regulation of endogenous *NEAT1* levels using the CRISPR-Cas9 system in NSC-34 cells induces neurotoxicity. *NEAT1_2*, but not *NEAT1_1*, was proposed to mediate this neurotoxic effect (Suzuki et al., 2019). These data support the idea that altered *NEAT1* expression in ALS leads to defects in paraspeckle formation causing cell death and neurodegeneration (Figure 1c). However, a neuroprotective role for *NEAT1* is proposed in Parkinson's disease (PD) (Simchovitz et al., 2019). Increased *NEAT1* levels were found in the substantia nigra of PD patients and in drug-induced oxidative stress in vitro PD models. This increase in expression was also associated with formation of *NEAT1* paraspeckles both in vitro and in vivo. Further, neuroprotective agents, like fenofibrate and simvastatin, were found to induce *NEAT1* expression, whereas *NEAT1*

depletion increased oxidative stress-induced cell death (Simchovitz et al., 2019).

Several of the genes associated with ALS give rise to sense or antisense RNAs which are not translated into protein but act as lncRNAs. These include naturally occurring (anti)sense RNAs but also RNAs that gain lncRNA functions as a result of genetic changes (e.g. repeat expansions). A few prominent examples are discussed below.

3.3 | *C9ORF72*

As indicated in previous sections, the expansion of a repeat region of six-nucleotide motifs $(GGGGCC)_n$ (G_4C_2) in the 5' region of the *C9ORF72* gene is the most common genetic cause of ALS and frontotemporal dementia (FTD; DeJesus-Hernandez et al., 2011; Renton et al., 2011; Zu et al., 2013). FTD is a form of dementia characterized by impaired judgement and executive skills, and loss of neurons in the frontal and temporal cortices (Burrell et al., 2016). ALS and FTD show overlapping disease phenotypes both at the symptomatic and genetic levels. The (G_4C_2) repeat expansions in *C9ORF72* are observed in three transcript variants: in variants 1 and 3 the expansions are located in intron 1 and in variant 2 they are present in the promoter region (Balendra & Isaacs, 2018). These *C9ORF72* repeat expansions are not only found in almost 40% of familial ALS and FTD cases, but also in up to 8% of sporadic cases of ALS and FTD (DeJesus-Hernandez et al., 2011; Majounie et al., 2012; Rademakers, 2012; Renton et al., 2011). Healthy individuals carry up to 20–30 copies of the repeat, whereas repeat size increases to hundreds to thousands of (G_4C_2) sequences in ALS patients (DeJesus-Hernandez et al., 2011). *C9ORF72* loss-of-function and toxic gain-of-function mediated by the repeat expansions have been implicated in *C9ORF72*-associated ALS cases (C9-ALS).

In addition to different sense isoforms of *C9ORF72*, one or more antisense transcripts that arise from the same promoter have been detected. The mutant hexanucleotide repeat regions of *C9ORF72* are transcribed in a bidirectional fashion, producing sense (*C9ORF72-S*), with G_4C_2 repeats, and antisense (*C9ORF72-AS*), with $GGCCCC$ (G_2C_4) repeats, RNAs (Donnelly et al., 2013; Mizielińska et al., 2013; Mori, et al., 2013; Renton et al., 2011). Both expanded repeat-containing transcripts (sense and antisense) can be translated into poly-dipeptides (dipeptide repeat [DPR] proteins) which accumulate in MNs of C9-ALS patients (Figure 1c; Freibaum & Taylor, 2017; Gendron et al., 2017; Mori, et al., 2013; Saberi et al., 2018; Wen et al., 2014; Zu et al., 2013). Several studies have focused on understanding the function of *C9ORF72-S* RNA and its associated proteins (reviewed in Moens et al., (2017)). Over-expression of G_4C_2 repeats in cell culture, *C. elegans*, *Danio rerio* and *Drosophila melanogaster* led to deleterious phenotypes, which provided evidence that *C9ORF72*-associated toxic gain-of-function contributed to the disease (Burguete et al., 2015; Freibaum et al., 2015; Kramer et al., 2016; Lee et al., 2013; Mizielińska et al., 2014; Wen et al., 2014; Xu et al., 2013; Zu et al., 2011). Similarly, adeno-associated virus-mediated over-expression of 66 G_4C_2 repeats in mice led



to an accumulation of RNA foci and DPR proteins together with neuronal cell loss, TDP-43 pathology and cognitive and motor dysfunction (Chew et al., 2015). The functional relevance of *C9ORF72-AS* is less well understood. Interestingly, *C9ORF72-AS* RNAs harbour a few miRNA-binding sites in the first exon (Douglas, 2018). Similar to *C9ORF72-S*, *C9ORF72-AS* forms RNA foci in MNs and sporadically in interneurons (in frontal cortex and spinal cord) of ALS and FTD patients (DeJesus-Hernandez et al., 2011; Gendron et al., 2013; Mizielinska et al., 2013; Zu et al., 2013). However, a higher frequency of *C9ORF72-AS* as compared to *C9ORF72-S* RNA foci and DPR proteins is observed in MNs of C9-ALS patients (Cooper-Knock et al., 2015). *C9ORF72-AS* RNA foci are found in the peri-nucleolar regions, suggesting that nucleolar defects or stress may contribute to C9-ALS pathogenesis (Kwon et al., 2014; Tao et al., 2015). It is important to note, however, that RNA foci formed by either *C9ORF72-S* or *C9ORF72-AS* do not correlate with the extent of neurodegeneration observed in ALS patients and are not determining factors of the clinico-pathological variability observed in C9-ALS cases (DeJesus-Hernandez et al., 2017). This may imply a role for other toxic modifiers in disease pathogenesis.

C9ORF72-S expansions are reported to form G-quadruplexes, which regulate transcription and gene expression (FratTA et al., 2012; Haeusler et al., 2014; Reddy et al., 2013; Wang, Goodrich, et al., 2019). *C9ORF72-AS* repeats do not form G-quadruplexes but can form i-Motifs and hairpin structures, that can affect genome stability and transcription (Assi et al., 2018; Kovanda et al., 2015). Further, the crystal structure of *C9ORF72-AS* repeat RNA showed that *C9ORF72-AS* RNA forms a double helix structure with tandem C:C mismatches, that could attract proteins recognizing these structures (Dodd et al., 2016). Therefore, repeat expansions in *C9ORF72* may not only affect ALS pathogenesis through the formation of RNA foci or production of DPR proteins, but also indirectly by regulating the secondary structure of DNA and RNA, and thereby gene expression.

To determine how ALS-associated *C9ORF72* repeat expansions cause ALS, different *in vivo* models have been generated. Expression of *C9ORF72* repeat expansions that form DPR proteins in *Drosophila* is toxic leading to adult-onset neurodegeneration with reduced lifespan (Mizielinska et al., 2014). Expression of repeat expansion RNA that is not translated into DPR proteins did not induce neurotoxicity, suggesting that the toxic effect of *C9ORF72* repeat expansions may be mediated by DPR proteins (Mizielinska et al., 2014). To better understand the role of *C9ORF72-S* and *C9ORF72-AS* repeat RNA expansions, another study created RNA-only *Drosophila* models, to avoid the effects of DPR proteins. Flies were generated to express 1) small (~100) RNA repeats in sense or antisense directions either as a processed polyadenylated transcript (named polyA) or as intronic versions fused with green fluorescent protein exons, or 2) large RNA-only repeats (>1,000) in the sense direction (Moens et al., 2018). Both, sense and antisense polyA repeat RNA was found to accumulate in the cytoplasm of adult *Drosophila* neurons, whereas intronic RNAs with small and large repeats accumulated in the nucleus forming RNA foci, mimicking pathological conditions. Even

though the RNA foci sequestered endogenous *Drosophila* RBPs, no neurodegenerative phenotypes or toxicity were observed following either sense or antisense RNA expression (small and large repeats). This suggests that mutant RNA is not sufficient to induce disease-relevant toxicity (Moens et al., 2018). However, expression of repeat expansions in a *Danio rerio* (zebrafish) model induced neurodegenerative phenotypes (Swinnen et al., 2018). Injection of ~70 *C9ORF72-S* (G₄C₂) or *C9ORF72-AS* (G₂C₄) repeats in zebrafish embryos induced motor axonopathy. A dose-dependent effect was observed on axon outgrowth and branching. Further, it was shown that toxicity induced by repeat RNA was independent of DPR proteins, suggesting that the presence of repeat RNA in the absence of DPR proteins is sufficient to induce MN toxicity in zebrafish (Swinnen et al., 2018). These differences in sensitivity for RNA-induced toxicity may be explained by species differences or differences in experimental approaches. However, even within one species (mouse) different effects of the expression of repeat RNAs were found. Even though in most models RNA foci and DPR protein inclusions formed from both sense and antisense *C9ORF72* RNAs were observed, some models developed motor or cognitive defects, whereas others were unaffected (Jiang, Zhu, et al., 2016; Liu et al., 2016; O'Rourke et al., 2015; Peters et al., 2015). Further work is needed to carefully assess the role of RNA toxicity in C9-ALS. Expression of longer repeat sequences (in the pathological range) may be required to model RNA toxicity accurately.

Despite this recent progress, the precise effects of the RNA foci or DPR proteins observed in C9-ALS are still unclear. Although valuable, studies on post-mortem tissue from patients do not reveal early pathogenic events and experimental work provides conflicting conclusions. The inconsistencies in recapitulating toxicity observed in ALS *C9ORF72* gain-of-function models could be because of the: (a) methodological difficulties in cloning and expressing GC-rich repeats at a length that is observed in patients leading to inconsistent mimicking of repeat pathology; (b) over-expression of repeat expansions in different *in vitro* and *in vivo* models, which may not necessarily mimic the endogenous levels of the expanded *C9ORF72-S* or *-AS* transcripts observed in patients; and (c) sequestration of a different set of RBPs by RNA foci in humans and different experimental models (Balendra & Isaacs, 2018).

Targeting of *C9ORF72-S* by antisense oligonucleotides (ASOs) and of the G-quadruplex structure formed by *C9ORF72-S* by specific binding molecules have been explored as therapeutic strategies for C9-ALS (Donnelly et al., 2013; Jiang, Zhu, et al., 2016; Lagier-Tourenne et al., 2013; Simone et al., 2018; Zamiri et al., 2014; Zhang et al., 2015). ASOs were effective in reducing RNA foci and reversed gene expression changes in C9-ALS hiPSC-derived MNs (Sareen et al., 2013). Even specific targeting of the repeat expansion by ASOs mitigated pathological hallmarks of ALS. In addition to reducing intranuclear accumulation of *C9ORF72-S* in RNA foci and normalizing dysregulated gene expression, ASOs were effective in reducing sequestration of the RBP adenosine deaminase RNA-specific B2 (inactive) to the repeat expansion and in reversing glutamate toxicity in C9-ALS patient fibroblasts and neurons (Donnelly et al., 2013).

However, ASOs targeting the sense strand were not effective in reducing the abundant RNA foci formed by *C9ORF72-AS* (Lagier-Tourenne et al., 2013). This could limit clinical efficacy if toxicity in patients is mainly caused by *C9ORF72-AS* RNA. Another important consideration for ASO therapy are off-target effects. The application of ASOs could lead to adverse side effects, because of their off-target effects depending on the sequence and modification chemistry of the motifs used to stabilize the ASO-molecule for protection against cellular degradation (Schoch & Miller, 2017). Off-target effects of ASOs are caused by binding to other mRNAs leading to an undesirable biological effect and activation of the immune system. Non-specific target binding is avoided by careful selection of candidate ASOs. Human clinical ASO candidates are routinely screened in silico for potential mismatches and tested empirically for their effect on mRNAs (Kamola et al., 2015; Monia et al., 1992). Activation of the immune system is because of the recognition of ASOs with certain chemical modifications as foreign DNA by toll-like receptors. It is reported that, unmethylated cytosine-phosphorus-guanine motifs in the ASO are recognized by toll-like receptors-9, resulting in antibody production, activation of T lymphocytes, natural killer cells and release of cytokines, interleukins (IL)-6, IL-12 and interferon (INF)- γ (Agrawal & Kandimalla, 2004; Karaki et al., 2019; Vollmer et al., 2004). However, adaptations in the ASO design have largely mitigated this effect (Bennett & Swayze, 2010; Scoles et al., 2019; Sewell et al., 2002). Despite extensive research in ASO development, unrecognized off-target issues remain (Schoch & Miller, 2017). So, it is important that each ASO molecule is evaluated independently and considered for a complete toxicological assessment.

Different effects of antisense transcripts on their sense counterparts have been described (Kanduri, 2008; Khorkova et al., 2014; Malecová & Morris, 2010; Modarresi et al., 2012; Yu et al., 2008). First, formation of double-stranded RNA structures by hybridization of overlapping complementary RNA sequences in sense and antisense transcripts can activate the RNA interference pathway (Poliikephad & Corry, 2013; Werner et al., 2014). Second, antisense transcripts may cause transcriptional interference via the displacement of sense transcription factors in the sense promoter region (Wight & Werner, 2013). Third, antisense transcripts may epigenetically regulate sense transcripts through recruitment of chromatin remodelling factors and modulation of their activity (Kanduri, 2008; Malecová & Morris, 2010; Yu et al., 2008). Given these possible roles of antisense transcripts, sense oligonucleotides (SOs) designed against antisense transcripts may be a valuable therapeutic strategy. However, further studies are needed to better define the role of *C9ORF72-AS* in C9-ALS.

3.4 | ATAXIN

The ALS risk gene *ataxin-2* (*ATXN2*) also gives rise to antisense transcripts, *ATXN2-AS*, with increased expression in ALS patient tissue (Li, Sun, et al., 2016). *ATXN2* protein is ubiquitously expressed in neuronal and non-neuronal tissues and is localized to the cytoplasm

(Huynh et al., 1999). *ATXN2* associates with polyribosomes in the endoplasmic reticulum and regulates mRNA translation (Satterfield & Pallanck, 2006). Further, it influences mRNA maturation, energy metabolism and endocytosis (Huynh et al., 1999; Magaña et al., 2013). In ALS, mutant *ATXN2* interacts with FUS and TDP-43 in an RNA-dependent interaction manner which contributes to disease pathogenesis (Elden et al., 2010; Van den Heuvel et al., 2014; Yokoshi et al., 2014). The interaction of *ATXN2* and TDP-43 was first identified in a screen for toxic modifiers of TDP-43. This work showed that an increase in the length of a polyQ repeat in *ATXN2* (from 22 normal to 27–33 glutamines in ALS) is significantly associated with enhanced risk for developing ALS (Elden et al., 2010). *ATXN2* polyQ repeat expansions of more than 33 repeats are associated with another neurodegenerative disease spinocerebellar ataxia type 2 (*SCA2*; Li, Sun, et al., 2016; Van den Heuvel et al., 2014). *SCA2* is an autosomal-dominant disorder caused by the expansion of CAG repeats in the N-terminal coding region of *ATXN2*. Clinical features of *SCA2* are characterized mainly by limb and gait ataxia, dysarthria and abnormal eye movements (Cancel et al., 1997; Geschwind et al., 1997). The symptoms of *SCA2* are found to be clearly of cerebellar origin and involve cerebellar circuits. However, a typical ocular-dependent feature is also observed in *SCA2*, where slow or absent saccades are displayed arising from the degeneration of oculomotor neurons. Other frequently observed symptoms include dystonia, myoclonus as well as neuropathy, muscle spasticity and frontal-executive dysfunction (Geschwind et al., 1997; Scoles & Pulst, 2018). The *ATXN2* locus is bidirectionally transcribed in both ALS and *SCA2* patients carrying an *ATXN2* repeat expansion. In three human ALS lymphoblastoid lines a non-translatable *ATXN2-AS* transcript was detected from both the normal and expanded allele (with intermediate CAG expansions [31–32 triplets]). Toxicity assays were performed to confirm the toxic nature of the expanded *ATXN2-AS* transcript in SK-N-MC neuroblastoma cells (Li, Sun, et al., 2016). These mutant CAG/CUG transcripts aberrantly interact with RBPs involved in, for example, ribosomal RNA (rRNA) processing and splicing (Figure 1c; Swinnen et al., 2020). Despite these promising results, whether and if so how *ATXN2-AS* contributes to ALS pathogenesis remains to be established.

3.5 | Other lncRNAs in ALS

Whole transcriptome RNA sequencing (RNA-seq) analysis in peripheral blood mononuclear cells (PBMCs) and spinal cord from sALS and fALS patients identified several differentially expressed lncRNAs (Gagliardi, et al., 2018). Several of these lncRNAs were antisense RNAs that were deregulated in sALS, for example, *ZEB1-AS* and *ZBTB11-AS*, and with sense counterparts that have reported functions in transcription regulation (Keightley et al., 2017; Li, Xie, et al., 2016). Interestingly, a few of these antisense lncRNAs can potentially target genes associated with neurodegenerative diseases, that is, *UBXN7-AS*, *ATG10-AS* and *ADORA2A-AS* (Guo & Qi, 2017; Lee et al., 2015; Villar-Menéndez et al., 2014). The detection of lncRNAs



in PBMCs and blood raises the possibility that lncRNAs may be used as biomarkers for assessing clinical disease course or characteristics. Intriguingly, several of the lncRNAs detected in healthy and diseased MNs have also been reported in blood, PBMCs or peripheral blood lymphocytes, for example, *NEAT1*, *MALAT1*, *MEG3* and *ZEB1-AS1* (Fallah et al., 2019; Gagliardi, et al., 2018; Gao et al., 2016; Huang et al., 2017; Wang, Li, et al., 2019).

Other lncRNAs have been implicated in ALS (or FTD) through their link with proteins associated with ALS pathogenesis, for example, TDP-43 or FUS. *MALAT1* showed increased expression and binding to TDP-43 in cortical tissue of sporadic FTD patients, while *Meg3* was down-regulated and showed decreased TDP-43 binding (Tollervey et al., 2011; Zhang et al., 2017). TDP-43 can associate with several other lncRNAs, including *BDNFOS* and *TFEB α* (in SHSY5Y cells) and *Myolinc* (in muscle cells; Militello et al., 2018; Xiao et al., 2011). Expression of the lncRNA *heat-shock rna ω* (*hsr ω*) is positively regulated by TDP-43, with direct binding of TDP-43 to the *hsr ω* locus (Chung et al., 2018). The stress-induced lncRNA *Satellite III repeat RNA* (*Sat III*) is the human orthologue of *Drosophila hsr ω* and shows increased expression in human FTD patient tissue and in cellular models of TDP-43 over-expression (Chung et al., 2018).

The introduction of ALS-associated mutations in FUS in mESC-derived MNs affects the expression of several lncRNAs. A specific up-regulation of the lncRNA *Lhx1os* and down-regulation of *LncMN-1* (*2610316D01Rik*) and *LncMN-2* (*5330434G04Rik*) were observed in FUS^{P517L/517L} and FUS^{-/-} MNs, indicating that FUS loss-of-function affects lncRNA expression. *Lhx1os*, *LncMN-1* and *LncMN-2* are conserved between mouse and human, displaying increased expression during human in vitro MN differentiation (Biscarini et al., 2018). The *Drosophila* orthologue of human FUS, dFUS, interacts with *hsr ω* . Depletion of *hsr ω* leads to cytoplasmic mis-localization and loss of nuclear dFUS function. Further, MN-specific knockdown of *hsr ω* impairs locomotion in larval and adult flies, and also induces anatomical defects in pre-synaptic terminals of MNs (Lo Piccolo & Yamaguchi, 2017). Given these diverse interactions of TDP-43 and FUS with lncRNAs it is likely that the cytoplasmic mis-localization and perturbed functions of TDP-43 and FUS in ALS MNs impacts on lncRNA distribution, expression and/or function that may contribute to MN degeneration and ALS.

3.6 | Spinal muscular atrophy (SMA)

SMA is a neurodegenerative disease characterized by profound muscle weakness attributed to progressive spinal MN degeneration, observed within weeks to months after birth or even at birth. SMA is classified as a rare disease that has a worldwide incidence of one in 6,000 – 10,000 newborns. However, it is the second most common autosomal recessive disease after cystic fibrosis and is the most common monogenic defect leading to infant mortality (Lunn & Wang, 2016). SMA is caused by reduced levels of SMN protein because of recessive mutations of the *SMN1* gene and retention of a variable copy number of the highly homologous *SMN2* gene

(Lefebvre et al., 1995; Lorson et al., 1999). The critical sequence difference between *SMN2* and *SMN1* is one nucleotide (C \rightarrow T) in exon 7 at a splice enhancer site. This change results in alternative splicing of *SMN2* pre-mRNA (Lorson et al., 1999; Monani et al., 1999). This leads to a preferential exclusion of exon 7 from most transcripts (*SMN Δ 7*), leading to a truncated unstable SMN protein, contributing to reduced levels of overall functional SMN protein. Individuals affected by SMA show loss of SMN protein and carry at least one copy of *SMN2*. The number of *SMN2* copies inversely correlates with disease severity (Lefebvre et al., 1995). In addition to *SMN2* copy number, other modifiers are known to modulate SMA clinical severity (Crawford et al., 2012). Therapy development for SMA has focused on increasing SMN expression and, as outlined below, lncRNAs may be valuable targets for modulating SMN expression. A lncRNA that arises from the antisense strand of SMN, *SMN-AS1*, represses SMN expression (d'Ydewalle et al., 2017; Woo et al., 2017). Similar to what is described for *C9ORF72-AS* and *ATXN2-AS*, *SMN-AS1* is a naturally occurring antisense transcript. *SMN1-AS* mainly localizes to neural tissues and neurons, and its expression increases during neuronal differentiation, inversely correlating with SMN protein expression. Chromatin immunoprecipitation showed that *SMN1-AS* mediates the recruitment of PRC2 to the *SMN2* promoter, suppressing SMN protein expression. This effect can be rescued by knockdown of *SMN1-AS* or inhibition of PRC2 activity (d'Ydewalle et al., 2017; Woo et al., 2017). Targeting *SMN1-AS* using ASOs together with *SMN2* splice-switching oligonucleotides increases SMN expression in a dose-dependent manner and improves survival in a severe mouse model of SMA (d'Ydewalle et al., 2017). These studies provide an important proof-of-concept for targeting lncRNAs as a means of inducing SMN expression and modulating disease progression.

3.7 | CircRNAs in motor neuron disease

Several studies have investigated circRNAs in the context of human disease (Chen, Li, et al., 2016). In recent years, transcriptome analyses have revealed that dysregulation of circRNAs is associated with complex neurological diseases such as Alzheimer's disease, PD, multiple system atrophy and ALS (Chen, Mills, et al., 2016; D'Ambra et al., 2019; Errichelli et al., 2017; Hanan et al., 2020; Huang et al., 2018; Wang et al., 2018). As outlined in previous sections, FUS is required for circRNA biogenesis (Errichelli et al., 2017). hiPSC-derived MNs harbouring ALS-linked FUS mutations (FUS-P525L) also showed deregulation of specific circRNAs suggesting that these mutations affect circRNA biogenesis. CircRNAs may not only play a role in ALS pathogenesis but could also be exploited as therapeutic agents. A genome wide loss-of-function screen for modifiers of TDP-43 toxicity identified (intronic) lariats as decoys for TDP-43. Reduction of debranching enzyme 1 activity led to an increase in intronic lariats in this screen. The newly formed lariats acted as decoys and sequestered toxic cytoplasmic TDP-43 aggregates, possibly preventing them from interacting with other RNAs and RBPs (Armakola et al., 2012). This study suggests the possibility of using

circular lncRNAs (such as circRNAs) as decoys for toxic proteins in ALS. CircRNAs were also investigated as potential biomarkers in ALS using microarray analysis (Dolinar et al., 2019). Expression profiling of circRNAs in ALS was performed by comparing leukocyte samples from 12 patient and eight age- and sex-matched healthy controls. Interestingly, several of the dysregulated circRNAs were located in gene loci that play key roles in ALS pathologies, for example, *hsa_circ_0001173* in vesicle-associated membrane protein-associated protein B/C (Nishimura et al., 2004). Three of the circRNA candidates (*hsa_circ_0023919*, *hsa_circ_0063411* and *hsa_circ_0088036*) showed similar differential expression patterns in a replication experiment as in the original microarray experiment. Where, *hsa_circ_0023919* was down-regulated, *hsa_circ_0063411* and *hsa_circ_0088036* were up-regulated in ALS patients, with good sensitivity and specificity for detection. This suggests that these candidate circRNAs could be used as diagnostic markers for sALS (Dolinar et al., 2019). While microarrays only allow detection of selected RNAs, RNAseq approaches can facilitate a more unbiased detection of circRNAs in the future, including of low abundant and novel transcripts, and in identification of their biomarker potential.

Overall, these studies hint at roles for circRNAs in the pathogenesis of ALS, and as diagnostic and therapeutic targets.

4 | CONCLUSION

During the past years, a large body of experimental evidence has highlighted the role of lncRNAs in MN development. Up-regulation of the expression of linear and circular lncRNAs during MN development, conservation of these RNA molecules across species and their involvement in polycomb repressive complex 1/2 recruitment and function are common observations. Interestingly, often the same cluster of lncRNAs that is regulated during MN development is dysregulated in a MND context (e.g. *Lhx1os*, *lncMN-1*, *lncMN-2*). These results suggest that lncRNAs are essential for normal MN development and that their dysregulation could underlie the pathogenesis of diseases such as ALS and SMA. The main challenge in the field is to identify 'key' downstream targets that are affected by lncRNA dysregulation and that lead to MN degeneration. Future work should focus on downstream targets that go beyond the direct interactors of lncRNAs but that instead unveil underlying molecular networks. For this, development of both genetic tools and innovative in vitro/in vivo models is essential. Taking advantage of iPSC technology and genome editing for generating patient-derived models (and isogenic controls) will be the key for dissecting the different roles of lncRNAs. Knockdown approaches, co-immunoprecipitation and argonaute 2-CLIP experiments combined with proteomics and next-generation sequencing analysis of lncRNAs are part of the toolbox that could reveal the nature and consequences of lncRNA interactions. Such experimental strategies would allow the establishment of a direct link between lncRNA-related targets and functions and consequent phenotypic alterations in MNs. Such improved understanding of

the mechanism-of-action and roles of lncRNAs will open up exciting ideas for molecular strategies for treating MNDs. An interesting hypothesis that deserves further investigation is the idea that lncRNAs may contribute to the selective vulnerability of specific subtypes of MNs. Other classes of ncRNAs, including miRNAs, can mediate the subtype-specific resistance of MNs to degenerate (Hoye et al., 2017; Tung et al., 2019). For example, reduced expression of the *miR-17-92* cluster of miRNAs in limb innervating lateral motor column-motor neurons (LMC-MNs) is associated with increased vulnerability of LMC-MNs to degenerate (Kanning et al., 2010; Tung et al., 2019). lncRNAs may subserve similar functions, especially given their ability to influence miRNA function. Sex-specific expression of lncRNAs also has been reported and it is interesting from a disease perspective as MNDs such as ALS and X-linked SMA occur more frequently in males as compared to females (Dressman et al., 2007; Wijesekera & Leigh, 2009). Currently, not much is known about potential sex-specific expression of the lncRNAs but establishing such expression patterns would be the first step to probe sex-specific lncRNA functions in healthy and diseased MNs.

Overall, accumulating experimental evidence hints at important roles for lncRNAs in MN development and disease. Although in many instances further work is needed to dissect how lncRNAs function in developing MNs and why lncRNA dysregulation contributes to MNDs, it is clear that such knowledge could aid the development of diagnostic and therapeutic approaches for treating MN pathologies like ALS and SMA.

ACKNOWLEDGEMENTS

The authors thank Jan Veldink for critically reading the manuscript. This work was supported by the ALS Stichting Nederland (TOTALS, ALS-on-a-chip), the Dutch Epilepsiefonds (WAR 18-05) and the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 721890 (circRTrain ITN).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

ORCID

Vamshidhar R. Vangoor  <https://orcid.org/0000-0003-0751-9772>
 Andreia Gomes-Duarte  <https://orcid.org/0000-0001-6079-1906>
 R. Jeroen Pasterkamp  <https://orcid.org/0000-0003-1631-6440>

REFERENCES

- Agrawal, S., & Kandimalla, E. R. (2004). Antisense and siRNA as agonists of Toll-like receptors. *Nature Biotechnology*, 22(12), 1533–1537. <https://doi.org/10.1038/nbt1042>
- Albertson, D. N., Schmidt, C. J., Kapatos, G., & Bannon, M. J. (2006). Distinctive profiles of gene expression in the human nucleus accumbens associated with cocaine and heroin abuse. *Neuropsychopharmacology*, 31, 2304–2312. <https://doi.org/10.1038/sj.npp.1301089>



- Al-Chalabi, A., & Hardiman, O. (2013). The epidemiology of ALS: A conspiracy of genes, environment and time. *Nature Reviews. Neurology*, *9*, 617–628. <https://doi.org/10.1038/nrneurol.2013.203>
- Al-Chalabi, A., Hardiman, O., Kiernan, M. C., Chiò, A., Rix-Brooks, B., & van den Berg, L. H. (2016). Amyotrophic lateral sclerosis: Moving towards a new classification system. *The Lancet Neurology*, [https://doi.org/10.1016/S1474-4422\(16\)30199-5](https://doi.org/10.1016/S1474-4422(16)30199-5)
- An, H., Skelt, L., Notaro, A., Highley, J. R., Fox, A. H., La Bella, V., Buchman, V. L., & Shelkovernikova, T. A. (2019). ALS-linked FUS mutations confer loss and gain of function in the nucleus by promoting excessive formation of dysfunctional paraspeckles. *Acta Neuropathologica Communications*, *7*, 7. <https://doi.org/10.1186/s40478-019-0658-x>
- Armakola, M., Higgins, M. J., Figley, M. D., Barmada, S. J., Scarborough, E. A., Diaz, Z., Fang, X., Shorter, J., Krogan, N. J., Finkbeiner, S., Farese, R. V., & Gitler, A. D. (2012). Inhibition of RNA lariat debranching enzyme suppresses TDP-43 toxicity in ALS disease models. *Nature Genetics*, *44*, 1302–1309. <https://doi.org/10.1038/ng.2434>
- Assi, H. A., Garavís, M., González, C., & Damha, M. J. (2018). I-motif DNA: Structural features and significance to cell biology. *Nucleic Acids Research*, <https://doi.org/10.1093/nar/gky735>
- Balendra, R., & Isaacs, A. M. (2018). C9orf72-mediated ALS and FTD: Multiple pathways to disease. *Nature Reviews Neurology*, *14*, 544–558. <https://doi.org/10.1038/s41582-018-0047-2>
- Banerjee, A., Vest, K. E., Pavlath, G. K., & Corbett, A. H. (2017). Nuclear poly(A) binding protein 1 (PABPN1) and matrin3 interact in muscle cells and regulate RNA processing. *Nucleic Acids Research*, *45*, 10706–10725. <https://doi.org/10.1093/nar/gkx786>
- Barry, G., Briggs, J. A., Vanichkina, D. P., Poth, E. M., Beveridge, N. J., Ratnu, V. S., Nayler, S. P., Nones, K., Hu, J., Bredy, T. W., Nakagawa, S., Rigo, F., Taft, R. J., Cairns, M. J., Blackshaw, S., Wolvetang, E. J., & Mattick, J. S. (2014). The long non-coding RNA Gomafu is acutely regulated in response to neuronal activation and involved in schizophrenia-associated alternative splicing. *Molecular Psychiatry*, *19*, 486–494. <https://doi.org/10.1038/mp.2013.45>
- Basu, S., Müller, F., & Sanges, R. (2013). Examples of sequence conservation analyses capture a subset of mouse long non-coding RNAs sharing homology with fish conserved genomic elements. *BMC Bioinformatics*, *14*, S14. <https://doi.org/10.1186/1471-2105-14-S7-S14>
- Bennett, C. F., & Swayze, E. E. (2010). RNA targeting therapeutics: Molecular mechanisms of antisense oligonucleotides as a therapeutic platform. *Annual Review of Pharmacology and Toxicology*, *50*, 259–293. <https://doi.org/10.1146/annurev.pharmtox.010909.105654>
- Biscarini, S., Caputo, D., Peruzzi, G., Lu, L., Colantoni, A., Santini, T., Shneider, N. A., Caffarelli, E., Laneve, P., & Bozzoni, I. (2018). Characterization of the lncRNA transcriptome in mESC-derived motor neurons: Implications for FUS-ALS. *Stem Cell Res.*, *27*, 172–179. <https://doi.org/10.1016/j.scr.2018.01.037>
- Blokhuis, A. M., Groen, E. J. N., Koppers, M., Van Den Berg, L. H., & Pasterkamp, R. J. (2013). Protein aggregation in amyotrophic lateral sclerosis. *Acta Neuropathologica*, <https://doi.org/10.1007/s00401-013-1125-6>
- Bonnefond, A., Vaillant, E., Philippe, J., Skrobek, B., Lobbens, S., Yengo, L., Huyvaert, M., Cavé, H., Busiah, K., Scharfmann, R., Polak, M., Abdul-Rasoul, M., Froguel, P., & Vaxillaire, M. (2013). Transcription factor gene MNX1 is a novel cause of permanent neonatal diabetes in a consanguineous family. *Diabetes Metab.*, *39*, 276–280. <https://doi.org/10.1016/j.diabet.2013.02.007>
- Braconi, C., Kogure, T., Valeri, N., Huang, N., Nuovo, G., Costinean, S., Negrini, M., Miotto, E., Croce, C. M., & Patel, T. (2011). MicroRNA-29 can regulate expression of the long non-coding RNA gene MEG3 in hepatocellular cancer. *Oncogene*, *30*, 4750–4756. <https://doi.org/10.1038/ncr.2011.193>
- Briese, M., Saal, L., Appenzeller, S., Moradi, M., Baluapuri, A., & Sendtner, M. (2016). Whole transcriptome profiling reveals the RNA content of motor axons. *Nucleic Acids Research*, *44*, e33. <https://doi.org/10.1093/nar/gkv1027>
- Burguete, A. S., Almeida, S., Gao, F.-B., Kalb, R., Akins, M. R., & Bonini, N. M. (2015). GGGGCC microsatellite RNA is neuritically localized, induces branching defects, and perturbs transport granule function. *Elife*, *4*, <https://doi.org/10.7554/eLife.08881>
- Burk, K., & Pasterkamp, R. J. (2019). Disrupted neuronal trafficking in amyotrophic lateral sclerosis. *Acta Neuropathologica*, *137*, 859–877. <https://doi.org/10.1007/s00401-019-01964-7>
- Burrell, J. R., Halliday, G. M., Kril, J. J., Ittner, L. M., Götz, J., Kiernan, M. C., & Hodges, J. R. (2016). The frontotemporal dementia-motor neuron disease continuum. *Lancet*, [https://doi.org/10.1016/S0140-6736\(16\)00737-6](https://doi.org/10.1016/S0140-6736(16)00737-6)
- Cabili, M., Trapnell, C., Goff, L., Koziol, M., Tazon-Vega, B., Regev, A., & Rinn, J. L. (2011). Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes and Development*, *25*, 1915–1927. <https://doi.org/10.1101/gad.17446611>
- Cancel, G., Dürr, A., Didierjean, O., Imbert, G., Bürk, K., Lezin, A., Belal, S., Benomar, A., Abada-Bendib, M., Vial, C., Guimarães, J., Chneiweiss, H., Stevanin, G., Yvert, G., Abbas, N., Saudou, F., Lebre, A. S., Yahyaoui, M., Hentati, F., ... Brice, A. (1997). Molecular and clinical correlations in spinocerebellar ataxia 2: A study of 32 families. *Human Molecular Genetics*, *6*, 709–715. <https://doi.org/10.1093/hmg/6.5.709>
- Carninci, P., Kasukawa, T., Katayama, S., Gough, J., Frith, M. C., Maeda, N., Oyama, R., & Ravasi, T. (2005). Transcriptional landscape of the Mammalian Genome. *Science*, *309*, 1559–1563.
- Chaudhry, M. A. (2014). Small nucleolar RNA host genes and long non-coding RNA responses in directly irradiated and bystander cells. *Cancer Biotherapy and Radiopharmaceuticals*, *29*, 135–141. <https://doi.org/10.1089/cbr.2013.1574>
- Chaytow, H., Huang, Y. T., Gillingwater, T. H., & Faller, K. M. E. (2018). The role of survival motor neuron protein (SMN) in protein homeostasis. *Cellular and Molecular Life Sciences*, *75*, 3877–3894. <https://doi.org/10.1007/s00018-018-2849-1>
- Chen, B. J., Mills, J. D., Takenaka, K., Bliim, N., Halliday, G. M., & Janitz, M. (2016). Characterization of circular RNAs landscape in multiple system atrophy brain. *Journal of Neurochemistry*, *139*, 485–496. <https://doi.org/10.1111/jnc.13752>
- Chen, K. W., & Chen, J. A. (2020). Functional roles of long non-coding RNAs in motor neuron development and disease. *Journal of Biomedical Science*, *27*, 1–14. <https://doi.org/10.1186/s12929-020-00628-z>
- Chen, L. L. (2016). The biogenesis and emerging roles of circular RNAs. *Nature Reviews Molecular Cell Biology*, *17*, 205–211. <https://doi.org/10.1038/nrm.2015.32>
- Chen, L. L., & Carmichael, G. G. (2009). Altered nuclear retention of mRNAs containing inverted repeats in human embryonic stem cells: Functional role of a nuclear noncoding RNA. *Molecular Cell*, *35*, 467–478. <https://doi.org/10.1016/j.molcel.2009.06.027>
- Chen, W., & Schuman, E. (2016). Circular RNAs in brain and other tissues: A functional enigma. *Trends in Neurosciences*, *39*, 597–604. <https://doi.org/10.1016/j.tins.2016.06.006>
- Chen, Y., Li, C., Tan, C., & Liu, X. (2016). Circular RNAs: A new frontier in the study of human diseases. *Journal of Medical Genetics*, <https://doi.org/10.1136/jmedgenet-2016-103758>
- Cheng, W., Wang, S., Zhang, Z., Morgens, D. W., Hayes, L. R., Lee, S., Portz, B., Xie, Y., Nguyen, B. V., Haney, M. S., Yan, S., Dong, D., Coyne, A. N., Yang, J., Xian, F., Cleveland, D. W., Qiu, Z., Rothstein, J. D., Shorter, J., ... Sun, S. (2019). CRISPR-Cas9 screens identify the RNA helicase DDX3X as a repressor of C9ORF72 (GGGGCC)n

- repeat-associated non-AUG translation. *Neuron*, 104, 885–898.e8. <https://doi.org/10.1016/j.neuron.2019.09.003>
- Chew, J., Gendron, T. F., Prudencio, M., Sasaguri, H., Zhang, Y. J., Castanedes-Casey, M., Lee, C. W., Jansen-West, K., Kurti, A., Murray, M. E., Bieniek, K. F., Bauer, P. O., Whitelaw, E. C., Rousseau, L., Stankowski, J. N., Stetler, C., Daugherty, L. M., Perkinson, E. A., Desaro, P., ... Petrucelli, L. (2015). C9ORF72 repeat expansions in mice cause TDP-43 pathology, neuronal loss, and behavioral deficits. *Science*, 348, 1151–1154. <https://doi.org/10.1126/science.aaa9344>
- Chodroff, R. A., Goodstadt, L., Sirey, T. M., Oliver, P. L., Davies, K. E., Green, E. D., Molnár, Z., & Ponting, C. P. (2010). Long noncoding RNA genes: Conservation of sequence and brain expression among diverse amniotes. *Genome Biology*, 11, R72. <https://doi.org/10.1186/gb-2010-11-7-r72>
- Chujo, T., Yamazaki, T., & Hirose, T. (2016). Architectural RNAs (arcRNAs): A class of long noncoding RNAs that function as the scaffold of nuclear bodies. *Biochimica Et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1859(1), 139–146. <https://doi.org/10.1016/j.bbgrm.2015.05.007>
- Chujo, T., Yamazaki, T., Kawaguchi, T., Kurosaka, S., Takumi, T., Nakagawa, S., & Hirose, T. (2017). Unusual semi-extractability as a hallmark of nuclear body-associated architectural noncoding RNA *s*. *EMBO Journal*, 36, 1447–1462. <https://doi.org/10.15252/emj.201695848>
- Chung, C.-Y., Berson, A., Kennerdell, J. R., Sartoris, A., Unger, T., Porta, S., Kim, H.-J., Smith, E. R., Shilatfard, A., Van Deerlin, V., Lee, V. M. Y., Chen-Plotkin, A., & Bonini, N. M. (2018). Aberrant activation of non-coding RNA targets of transcriptional elongation complexes contributes to TDP-43 toxicity. *Nature Communications*, 9, 4406. <https://doi.org/10.1038/s41467-018-06543-0>
- Clemson, C. M., Hutchinson, J. N., Sara, S. A., Ensminger, A. W., Fox, A. H., Chess, A., & Lawrence, J. B. (2009). An Architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. *Molecular Cell*, 33, 717–726. <https://doi.org/10.1016/j.molcel.2009.01.026>
- Cooper-Knock, J., Higginbottom, A., Stopford, M. J., Highley, J. R., Ince, P. G., Wharton, S. B., Pickering-Brown, S., Kirby, J., Hautbergue, G. M., & Shaw, P. J. (2015). Antisense RNA foci in the motor neurons of C9ORF72-ALS patients are associated with TDP-43 proteinopathy. *Acta Neuropathologica*, 130, 63–75. <https://doi.org/10.1007/s00401-015-1429-9>
- Crawford, T. O., Paushkin, S. V., Kobayashi, D. T., Forrest, S. J., Joyce, C. L., Finkel, R. S., Kaufmann, P., Swoboda, K. J., Tiziano, D., Lomastro, R., Li, R. H., Trachtenberg, F. L., Plasterer, T., & Chen, K. S. (2012). Evaluation of SMN protein, transcript, and copy number in the biomarkers for spinal muscular atrophy (BforSMA) clinical study. *PLoS One*, 7, e33572. <https://doi.org/10.1371/journal.pone.0033572>
- D'Ambra, E., Caputo, D., & Morlando, M. (2019). Exploring the regulatory role of circular RNAs in neurodegenerative disorders. *International Journal of Molecular Sciences*, 20, 5477. <https://doi.org/10.3390/ijms20215477>
- d'Ydewalle, C., Ramos, D. M., Pyles, N. J., Ng, S. Y., Gorz, M., Pilato, C. M., Ling, K., Kong, L., Ward, A. J., Rubin, L. L., Rigo, F., Bennett, C. F., & Sumner, C. J. (2017). The antisense transcript SMN-AS1 regulates SMN expression and is a novel therapeutic target for spinal muscular atrophy. *Neuron*, 93, 66–79. <https://doi.org/10.1016/j.neuron.2016.11.033>
- Davis, I. J., Hsi, B. L., Arroyo, J. D., Vargas, S. O., Yeh, Y. A., Motyckova, G., Valencia, P., Perez-Atayde, A. R., Argani, P., Ladanyi, M., Fletcher, J. A., & Fisher, D. E. (2003). Cloning of an Alpha-TFEB fusion in renal tumors harboring the t(6;11)(p21;q13) chromosome translocation. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 6051–6056. <https://doi.org/10.1073/pnas.0931430100>
- DeJesus-Hernandez, M., Finch, N. C. A., Wang, X., Gendron, T. F., Bieniek, K. F., Heckman, M. G., Vasilevich, A., Murray, M. E., Rousseau, L., Weesner, R., Lucido, A., Parsons, M., Chew, J., Josephs, K. A., Parisi, J. E., Knopman, D. S., Petersen, R. C., Boeve, B. F., Graff-Radford, N. R., ... Rademakers, R. (2017). In-depth clinico-pathological examination of RNA foci in a large cohort of C9ORF72 expansion carriers. *Acta Neuropathologica*, 134, 255–269. <https://doi.org/10.1007/s00401-017-1725-7>
- DeJesus-Hernandez, M., Mackenzie, I. R., Boeve, B. F., Boxer, A. L., Baker, M., Rutherford, N. J., Nicholson, A. M., Finch, N. C. A., Flynn, H., Adamson, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G. Y. R., Karydas, A., Seeley, W. W., Josephs, K. A., Coppola, G., Geschwind, D. H., ... Rademakers, R. (2011). Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron*, 72, 245–256. <https://doi.org/10.1016/j.neuron.2011.09.011>
- Derrien, T., Johnson, R., Bussotti, G., Tanzer, A., Djebali, S., Tilgner, H., Guernec, G., Martin, D., Merkel, A., Knowles, D. G., Lagarde, J., Veeravalli, L., Ruan, X., Ruan, Y., Lassmann, T., Carninci, P., Brown, J. B., Lipovich, L., Gonzalez, J. M., ... Guigó, R. (2012). The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. *Genome Research*, 22, 1775–1789. <https://doi.org/10.1101/gr.132159.111>
- Dinger, M. E., Amara, P. P., Mercer, T. R., Pang, K. C., Bruce, S. J., Gardiner, B. B., Askarian-Amiri, M. E., Ru, K., Soldà, G., Simons, C., Sunkin, S. M., Crowe, M. L., Grimmond, S. M., Perkins, A. C., & Mattick, J. S. (2008). Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. *Genome Research*, 18, 1433–1445. <https://doi.org/10.1101/gr.078378.108>
- Djebali, S., Davis, C. A., Merkel, A., Dobin, A., Lassmann, T., Mortazavi, A., Tanzer, A., Lagarde, J., Lin, W., Schlesinger, F., Xue, C., Marinov, G. K., Khatun, J., Williams, B. A., Zaleski, C., Rozowsky, J., Röder, M., Kokocinski, F., Abdelhamid, R. F., ... Gingeras, T. R. (2012). Landscape of transcription in human cells. *Nature*, 489, 101–108. <https://doi.org/10.1038/nature11233>
- Dodd, D. W., Tomchick, D. R., Corey, D. R., & Gagnon, K. T. (2016). Pathogenic C9ORF72 antisense repeat RNA forms a double helix with tandem C: C mismatches. *Biochemistry*, 55, 1283–1286. <https://doi.org/10.1021/acs.biochem.6b00136>
- Dolinar, A., Koritnik, B., Glavač, D., & Ravnik-Glavač, M. (2019). Circular RNAs as potential blood biomarkers in amyotrophic lateral sclerosis. *Molecular Neurobiology*, 56, 8052–8062. <https://doi.org/10.1007/s12035-019-1627-x>
- Donnelly, C. J., Zhang, P. W., Pham, J. T., Heusler, A. R., Mistry, N. A., Vidensky, S., Daley, E. L., Poth, E. M., Hoover, B., Fines, D. M., Maragakis, N., Tienari, P. J., Petrucelli, L., Traynor, B. J., Wang, J., Rigo, F., Bennett, C. F., Blackshaw, S., Sattler, R., & Rothstein, J. D. (2013). RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. *Neuron*, 80, 415–428. <https://doi.org/10.1016/j.neuron.2013.10.015>
- Douglas, A. G. L. (2018). Non-coding RNA in C9orf72-related amyotrophic lateral sclerosis and frontotemporal dementia: A perfect storm of dysfunction. *Non-coding RNA Research*, 3, 178–187. <https://doi.org/10.1016/j.ncrna.2018.09.001>
- Dressman, D., Ahearn, M. E., Yarz, K. O., Basterrecha, H., Martínez, F., Palau, F., Barmada, M. M., Clark, R. D., Meindl, A., Wirth, B., Hoffman, E. P., & Baumbach-Reardon, L. (2007). X-linked infantile spinal muscular atrophy: Clinical definition and molecular mapping. *Genetics in Medicine*, 9, 52–60. <https://doi.org/10.1097/GIM.0b013e31802d8353>
- Dunham, I., Kundaje, A., Aldred, S. F., Collins, P. J., Davis, C. A., Doyle, F., Epstein, C. B., Frietze, S., Harrow, J., Kaul, R., Khatun, J., Lajoie, B. R., Landt, S. G., Lee, B. K., Pauli, F., Rosenbloom, K. R., Sabo, P., Safi, A., Sanyal, A., ... Lochovsky, L. (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature*, 489, 57–74. <https://doi.org/10.1038/nature11247>
- Ebbesen, K. K., Kjems, J., & Hansen, T. B. (2016). Circular RNAs: Identification, biogenesis and function. *Biochimica Et Biophysica*



- Acta (BBA)-Gene Regulatory Mechanisms*, 1859, 163–168. <https://doi.org/10.1016/j.bbagr.2015.07.007>
- Elden, A. C., Kim, H. J., Hart, M. P., Chen-Plotkin, A. S., Johnson, B. S., Fang, X., Armakola, M., Geser, F., Greene, R., Lu, M. M., Padmanabhan, A., Clay-Falcone, D., McCluskey, L., Elman, L., Juhr, D., Gruber, P. J., Rüb, U., Auburger, G., Trojanowski, J. Q., ... Gitler, A. D. (2010). Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature*, 466, 1069–1075. <https://doi.org/10.1038/nature09320>
- Elling, R., Chan, J., & Fitzgerald, K. A. (2016). Emerging role of long non-coding RNAs as regulators of innate immune cell development and inflammatory gene expression. *European Journal of Immunology*, 46, 504–512. <https://doi.org/10.1002/eji.201444558>
- Errichelli, L., Dini Modigliani, S., Laneve, P., Colantoni, A., Legnini, I., Capauto, D., Rosa, A., De Santis, R., Scarfò, R., Peruzzi, G., Lu, L., Caffarelli, E., Shneider, N. A., Morlando, M., & Bozzoni, I. (2017). FUS affects circular RNA expression in murine embryonic stem cell-derived motor neurons. *Nature Communications*, 8, 1–11. <https://doi.org/10.1038/ncomms14741>
- Fallah, H., Azari, I., Neishabouri, S. M., Oskoeei, V. K., Taheri, M., & Ghafouri-Fard, S. (2019). Sex-specific up-regulation of lncRNAs in peripheral blood of patients with schizophrenia. *Scientific Reports*, 9, 1–8. <https://doi.org/10.1038/s41598-019-49265-z>
- Fallini, C., Bassell, G. J., & Rossoll, W. (2012). Spinal muscular atrophy: The role of SMN in axonal mRNA regulation. *Brain Research*, 1462, 81–92. <https://doi.org/10.1016/j.brainres.2012.01.044>
- Ferraiuolo, L., Meyer, K., Sherwood, T. W., Vick, J., Likhite, S., Frakes, A., Miranda, C. J., Braun, L., Heath, P. R., Pineda, R., Beattie, C. E., Shaw, P. J., Askwith, C. C., McTigue, D., & Kaspar, B. K. (2016). Oligodendrocytes contribute to motor neuron death in ALS via SOD1-dependent mechanism. *Proceedings of the National Academy of Sciences*, 113, E6496–E6505. <https://doi.org/10.1073/pnas.1607496113>
- Field, A. R., Jacobs, F. M. J., Fiddes, I. T., Phillips, A. P. R., Reyes-Ortiz, A. M., LaMontagne, E., Whitehead, L., Meng, V., Rosenkrantz, J. L., Olsen, M., Hauessler, M., Katzman, S., Salama, S. R., & Haussler, D. (2019). Structurally conserved primate lncRNAs are transiently expressed during human cortical differentiation and influence cell-type-specific genes. *Stem Cell Reports*, 12, 245–257. <https://doi.org/10.1016/j.stemcr.2018.12.006>
- Fox, A. H., & Lamond, A. I. (2010). Paraspeckles. *Cold Spring Harbor Perspectives in Biology*, 2, a000687. <https://doi.org/10.1101/cshpe.rspect.a000687>
- Fox, A. H., Nakagawa, S., Hirose, T., & Bond, C. S. (2018). Paraspeckles: Where long noncoding RNA meets phase separation. *Trends in Biochemical Sciences*, 43(2), 124–135. <https://doi.org/10.1016/j.tibs.2017.12.001>
- Fratta, P., Mizielińska, S., Nicoll, A. J., Zloh, M., Fisher, E. M. C., Parkinson, G., & Isaacs, A. M. (2012). C9orf72 hexanucleotide repeat associated with amyotrophic lateral sclerosis and frontotemporal dementia forms RNA G-quadruplexes. *Scientific Reports*, 2, 1016. <https://doi.org/10.1038/srep01016>
- Freibaum, B. D., Lu, Y., Lopez-Gonzalez, R., Kim, N. C., Almeida, S., Lee, K. H., Badders, N., Valentine, M., Miller, B. L., Wong, P. C., Petrucelli, L., Kim, H. J., Gao, F. B., & Taylor, J. P. (2015). GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. *Nature*, 525, 129–133. <https://doi.org/10.1038/nature14974>
- Freibaum, B. D., & Taylor, J. P. (2017). The role of dipeptide repeats in C9orf72-related ALS-FTD. *Frontiers in Molecular Neuroscience*, 10, <https://doi.org/10.3389/fnmol.2017.00035>
- Gabanello, F., Carissimi, C., Usiello, A., & Pellizzoni, L. (2005). The activity of the spinal muscular atrophy protein is regulated during development and cellular differentiation. *Human Molecular Genetics*, 14, 3629–3642. <https://doi.org/10.1093/hmg/ddi390>
- Gagliardi, S., Pandini, C., Garofalo, M., Bordoni, M., Pansarasa, O., & Cereda, C. (2018). Long non coding RNAs and ALS: Still much to do. *Non-coding RNA Research*, 3, 226–231. <https://doi.org/10.1016/j.ncrna.2018.11.004>
- Gagliardi, S., Zucca, S., Pandini, C., Diamanti, L., Bordoni, M., Sproviero, D., Arigoni, M., Olivero, M., Pansarasa, O., Ceroni, M., Calogero, R., & Cereda, C. (2018). Long non-coding and coding RNAs characterization in peripheral blood mononuclear cells and spinal cord from amyotrophic lateral sclerosis patients. *Scientific Reports*, 8, 1–11. <https://doi.org/10.1038/s41598-018-20679-5>
- Gao, C., He, Z., Li, J., Li, X., Bai, Q., Zhang, Z., Zhang, X., Wang, S., Xiao, X., Wang, F., Yan, Y., Li, D., Chen, L., Zeng, X., Xiao, Y., Dong, G., Zheng, Y., Wang, Q., & Chen, W. (2016). Specific long non-coding RNAs response to occupational PAHs exposure in coke oven workers. *Toxicology Reports*, 3, 160–166. <https://doi.org/10.1016/j.toxrep.2015.12.011>
- Gao, T., Li, J., Li, N., Gao, Y., Yu, L., Zhuang, S., Zhao, Y., & Dong, X. (2020). Lncrps25 play an essential role in motor neuron development through controlling the expression of Olig2 in zebrafish. *Journal of Cellular Physiology*, 235, 3485–3496. <https://doi.org/10.1002/jcp.29237>
- Gendron, T. F., Bieniek, K. F., Zhang, Y. J., Jansen-West, K., Ash, P. E. A., Caulfield, T., Daugherty, L., Dunmore, J. H., Castanedes-Casey, M., Chew, J., Cosio, D. M., Van Blitterswijk, M., Lee, W. C., Rademakers, R., Boylan, K. B., Dickson, D. W., & Petrucelli, L. (2013). Antisense transcripts of the expanded C9orf72 hexanucleotide repeat form nuclear RNA foci and undergo repeat-associated non-ATG translation in c9FTD/ALS. *Acta Neuropathologica*, 126, 829–844. <https://doi.org/10.1007/s00401-013-1192-8>
- Gendron, T. F., Chew, J., Stankowski, J. N., Hayes, L. R., Zhang, Y.-J., Prudencio, M., Carlomagno, Y., Daugherty, L. M., Jansen-West, K., Perkerson, E. A., O'Raw, A., Cook, C., Pregel, L., Belzil, V., van Blitterswijk, M., Tabassian, L. J., Lee, C. W., Yue, M., Tong, J., ... Poletti, L. (2017). Poly(GP) proteins are a useful pharmacodynamic marker for C9orf72-associated amyotrophic lateral sclerosis. *Science Translational Medicine*, 9, eaai7866. <https://doi.org/10.1126/scitranslmed.aai7866>
- Geschwind, D. H., Perlman, S., Figueroa, C. P., Treiman, L. J., & Pulst, S. M. (1997). The prevalence and wide clinical spectrum of the spinocerebellar ataxia type 2 trinucleotide repeat in patients with autosomal dominant cerebellar ataxia. *American Journal of Human Genetics*, 60, 842–850.
- Giavazzi, A., Setola, V., Simonati, A., & Battaglia, G. (2006). Neuronal-specific roles of the survival motor neuron protein: Evidence from survival motor neuron expression patterns in the developing human central nervous system. *Journal of Neuropathology and Experimental Neurology*, <https://doi.org/10.1097/01.jnen.0000205144.54457.a3>
- Groen, E. J. N., Fumoto, K., Blokhuis, A. M., Engelen-Lee, J. Y., Zhou, Y., van den Heuvel, D. M. A., Koppers, M., van Diggelen, F., van Heest, J., Demmers, J. A. A., Kirby, J., Shaw, P. J., Aronica, E., Spliet, W. G. M., Veldink, J. H., van den Berg, L. H., & Pasterkamp, R. J. (2013). ALS-associated mutations in FUS disrupt the axonal distribution and function of SMN. *Human Molecular Genetics*, 22, 3690–3704. <https://doi.org/10.1093/hmg/ddt222>
- Guo, X., & Qi, X. (2017). VCP cooperates with UBXD1 to degrade mitochondrial outer membrane protein MCL1 in model of Huntington's disease. *Biochimica Et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1863, 552–559. <https://doi.org/10.1016/j.bbdis.2016.11.026>
- Guttman, M., Amit, I., Garber, M., French, C., Lin, M. F., Feldser, D., Huarte, M., Zuk, O., Carey, B. W., Cassady, J. P., Cabili, M. N., Jaenisch, R., Mikkelsen, T. S., Jacks, T., Hacohen, N., Bernstein, B. E., Kellis, M., Regev, A., Rinn, J. L., & Lander, E. S. (2009). Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature*, 458, 223–227. <https://doi.org/10.1038/nature07672>



- Haeusler, A. R., Donnelly, C. J., Periz, G., Simko, E. A. J., Shaw, P. G., Kim, M.-S., Maragakis, N. J., Troncoso, J. C., Pandey, A., Sattler, R., Rothstein, J. D., & Wang, J. (2014). C9orf72 nucleotide repeat structures initiate molecular cascades of disease. *Nature*, *507*(7491), 195–200. <https://doi.org/10.1038/nature13124>
- Hanan, M., Simchovitz, A., Yayon, N., Vaknine, S., Cohen-Fultheim, R., Karmon, M., Madrer, N., Rohrllich, T. M., Maman, M., Bennett, E. R., Greenberg, D. S., Meshorer, E., Levanon, E. Y., Soreq, H., & Kadener, S. (2020). A Parkinson's disease Circ RNA s Resource reveals a link between circ SLC 8A1 and oxidative stress. *EMBO Molecular Medicine*, *12*, e11942. <https://doi.org/10.15252/emmm.201911942>
- He, D., Wang, J., Lu, Y., Deng, Y., Zhao, C., Xu, L., Chen, Y., Hu, Y. C., Zhou, W., & Lu, Q. R. (2017). lncRNA functional networks in oligodendrocytes reveal stage-specific myelination control by an lncOL1/Suz12 complex in the CNS. *Neuron*, *93*, 362–378. <https://doi.org/10.1016/j.neuron.2016.11.044>
- Hennig, S., Kong, G., Mannen, T., Sadowska, A., Kobelke, S., Blythe, A., Knott, G. J., Iyer, S. S., Ho, D., Newcombe, E. A., Hosoki, K., Goshima, N., Kawaguchi, T., Hatters, D., Trinkle-Mulcahy, L., Hirose, T., Bond, C. S., & Fox, A. H. (2015). Prion-like domains in RNA binding proteins are essential for building subnuclear paraspeckles. *Journal of Cell Biology*, *210*, 529–539. <https://doi.org/10.1083/jcb.201504117>
- Hirose, T., Virnicchi, G., Tanigawa, A., Naganuma, T., Li, R., Kimura, H., Yokoi, T., Nakagawa, S., Bénard, M., Fox, A. H., & Pierron, G. (2014). NEAT1 long noncoding RNA regulates transcription via protein sequestration within subnuclear bodies. *Molecular Biology of the Cell*, *25*, 169–183. <https://doi.org/10.1091/mbc.E13-09-0558>
- Hoye, M. L., Koval, E. D., Wegener, A. J., Hyman, T. S., Yang, C., O'Brien, D. R., Miller, R. L., Cole, T., Schoch, K. M., Shen, T., Kunikata, T., Richard, J. P., Gutmann, D. H., Maragakis, N. J., Kordasiewicz, H. B., Dougherty, J. D., & Miller, T. M. (2017). MicroRNA profiling reveals marker of motor neuron disease in ALS models. *Journal of Neuroscience*, *37*, 5574–5586. <https://doi.org/10.1523/JNEUROSCI.3582-16.2017>
- Huang, J. L., Qin, M. C., Zhou, Y., Xu, Z. H., Yang, S., Zhang, F., Zhong, J., Liang, M.-K., Chen, B., Zhang, W.-Y., Wu, D.-P., & Zhong, Z.-G. (2018). Comprehensive analysis of differentially expressed profiles of Alzheimer's disease associated circular RNAs in an Alzheimer's disease mouse model. *Aging (Albany, NY)*, *10*, 253–265. <https://doi.org/10.18632/aging.101387>
- Huang, S., Qian, K., Zhu, Y., Huang, Z., Luo, Q., & Qing, C. (2017). Diagnostic value of the lncRNA NEAT1 in peripheral blood mononuclear cells of patients with sepsis. *Disease Markers*, *2017*, 1–6. <https://doi.org/10.1155/2017/7962836>
- Huynh, D. P., Del Bigio, M. R., Ho, D. H., & Pulst, S. M. (1999). Expression of ataxin-2 in brains from normal individuals and patients with Alzheimer's disease and spinocerebellar ataxia 2. *Annals of Neurology*, *45*, 232–241. [https://doi.org/10.1002/1531-8249\(199902\)45:2<232:AID-ANA14>3.0.CO;2-7](https://doi.org/10.1002/1531-8249(199902)45:2<232:AID-ANA14>3.0.CO;2-7)
- Jeck, W. R., Sorrentino, J. A., Wang, K., Slevin, M. K., Burd, C. E., Liu, J., Marzluff, W. F., & Sharpless, N. E. (2013). Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA*, *19*, 141–157. <https://doi.org/10.1261/rna.035667.112>
- Jiang, C., Li, Y., Zhao, Z., Lu, J., Chen, H., Ding, N., Wang, G., Xu, J., & Li, X. (2016). Identifying and functionally characterizing tissue-specific and ubiquitously expressed human lncRNAs. *Oncotarget*, *7*, 7120–7133. <https://doi.org/10.18632/oncotarget.6859>
- Jiang, J., Zhu, Q., Gendron, T. F., Saberi, S., McAlonis-Downes, M., Seelman, A., Stauffer, J. E., Jafar-nejad, P., Drenner, K., Schulte, D., Chun, S., Sun, S., Ling, S. C., Myers, B., Engelhardt, J., Katz, M., Baughn, M., Platoshyn, O., Marsala, M., ... Lagier-Tourenne, C. (2016). Gain of toxicity from ALS/FTD-linked repeat expansions in C9ORF72 is alleviated by antisense oligonucleotides targeting GGGGCC-containing RNAs. *Neuron*, *90*, 535–550. <https://doi.org/10.1016/j.neuron.2016.04.006>
- Johansson, P., Lipovich, L., Grandér, D., & Morris, K. V. (2014). Evolutionary conservation of long non-coding RNAs; sequence, structure, function. *Biochimica Et Biophysica Acta (BBA) - General Subjects*, *1840*(3), 1063–1071. <https://doi.org/10.1016/j.bbagen.2013.10.035>
- Kamola, P. J., Kitson, J. D. A., Turner, G., Maratou, K., Eriksson, S., Panjwani, A., Warnock, L. C., Douillard Guilloux, G. A., Moores, K., Koppe, E. L., Wixted, W. E., Wilson, P. A., Gooderham, N. J., Gant, T. W., Clark, K. L., Hughes, S. A., Edbrooke, M. R., & Parry, J. D. (2015). In silico and in vitro evaluation of exonic and intronic off-target effects form a critical element of therapeutic ASO gapmer optimization. *Nucleic Acids Research*, *43*, 8638–8650. <https://doi.org/10.1093/nar/gkv857>
- Kanduri, C. (2008). Functional insights into long antisense noncoding RNA Kcnq1ot1 mediated bidirectional silencing. *RNA Biology*, *5*, 208–211. <https://doi.org/10.4161/rna.7113>
- Kanning, K. C., Kaplan, A., & Henderson, C. E. (2010). Motor neuron diversity in development and disease. *Annual Review of Neuroscience*, *33*, 409–440. <https://doi.org/10.1146/annurev.neuro.051508.135722>
- Karaki, S., Paris, C., & Rocchi, P. (2019). Antisense Oligonucleotides, A Novel Developing Targeting Therapy, in: Antisense Therapy. IntechOpen. <https://doi.org/10.5772/intechopen.82105>
- Keightley, M.-C., Carradice, D. P., Layton, J. E., Pase, L., Bertrand, J. Y., Wittig, J. G., Dakic, A., Badrock, A. P., Cole, N. J., Traver, D., Nutt, S. L., McCoe, J., Buckle, A. M., Heath, J. K., & Lieschke, G. J. (2017). The Pu.1 target gene Zbtb11 regulates neutrophil development through its integrase-like HHCC zinc finger. *Nature Communications*, *8*, 14911. <https://doi.org/10.1038/ncomms14911>
- Khorkova, O., Myers, A. J., Hsiao, J., & Wahlestedt, C. (2014). Natural antisense transcripts. *Human Molecular Genetics*, *23*, R54–R63. <https://doi.org/10.1093/hmg/ddu207>
- Kovanda, A., Zalar, M., Šket, P., Plavec, J., & Rogelj, B. (2015). Anti-sense DNA d(GGCCCC)n expansions in C9ORF72 form i-motifs and protonated hairpins. *Scientific Reports*, *5*, 3–9. <https://doi.org/10.1038/srep17944>
- Kramer, N. J., Carlomagno, Y., Zhang, Y. J., Almeida, S., Cook, C. N., Gendron, T. F., Prudencio, M., Van Blitterswijk, M., Belzil, V., Couthouis, J., Paul, J. W., Goodman, L. D., Daugherty, L., Chew, J., Garrett, A., Pregent, L., Jansen-West, K., Tabassian, L. J., Rademakers, R., ... Gitler, A. D. (2016). Spt4 selectively regulates the expression of C9orf72 sense and antisense mutant transcripts. *Science*, *353*(6300), 708–712. <https://doi.org/10.1126/science.aaf7791>
- Kung, J. T. Y., Colognori, D., & Lee, J. T. (2013). Long noncoding RNAs: Past, present, and future. *Genetics*, *193*(3), 651–669. <https://doi.org/10.1534/genetics.112.146704>
- Kwon, I., Xiang, S., Kato, M., Wu, L., Theodoropoulos, P., Wang, T., Kim, J., Yun, J., Xie, Y., & McKnight, S. L. (2014). Poly-dipeptides encoded by the C9orf72 repeats bind nucleoli, impede RNA biogenesis, and kill cells. *Science*, *345*, 1139–1145. <https://doi.org/10.1126/science.1254917>
- Lagier-Tourenne, C., Baughn, M., Rigo, F., Sun, S., Liu, P., Li, H. R., Jiang, J., Watt, A. T., Chun, S., Katz, M., Qiu, J., Sun, Y., Ling, S. C., Zhu, Q., Polymenidou, M., Drenner, K., Artates, J. W., McAlonis-Downes, M., Markmiller, S., ... Ravits, J. (2013). Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. *Proceedings of the National Academy of Sciences*, *110*, <https://doi.org/10.1073/pnas.1318835110>
- Lee, J. K., Shin, J. H., Lee, J. E., & Choi, E. J. (2015). Role of autophagy in the pathogenesis of amyotrophic lateral sclerosis. *Biochimica Et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1852*(11), 2517–2524. <https://doi.org/10.1016/j.bbadis.2015.08.005>
- Lee, Y. B., Chen, H. J., Peres, J. N., Gomez-Deza, J., Attig, J., Štalekar, M., Troakes, C., Nishimura, A. L., Scotter, E. L., Vance, C., Adachi, Y., Sardone, V., Miller, J. W., Smith, B. N., Gallo, J. M., Ule, J., Hirth, F.,



- Rogelj, B., Houart, C., & Shaw, C. E. (2013). Hexanucleotide repeats in ALS/FTD form length-dependent RNA foci, sequester RNA binding proteins, and are neurotoxic. *Cell Reports*, 5, 1178–1186. <https://doi.org/10.1016/j.celrep.2013.10.049>
- Lefebvre, S., Bürglen, L., Reboullet, S., Clermont, O., Burlet, P., Viollet, L., Benichou, B., Cruaud, C., Millasseau, P., Zeviani, M., Le Paslier, D., Frézal, J., Cohen, D., Weissenbach, J., Munnich, A., & Melki, J. (1995). Identification and characterization of a spinal muscular atrophy-determining gene. *Cell*, 80, 155–165. [https://doi.org/10.1016/0092-8674\(95\)90460-3](https://doi.org/10.1016/0092-8674(95)90460-3)
- Lelli, A., Nolan, K., Santambrogio, S., Marti, H., Hoogewijs, D., Frew, I., Goncalves, A. F., Schonenberger, M., Guinot, A., & Wenger, R. (2015). Induction of long noncoding RNA MALAT1 in hypoxic mice. *Hypoxia*, 3, 45. <https://doi.org/10.2147/HP.S90555>
- Li, P. P., Sun, X., Xia, G., Arbez, N., Paul, S., Zhu, S., Peng, H. B., Ross, C. A., Koeppen, A. H., Margolis, R. L., Pulst, S. M., Ashizawa, T., & Rudnicki, D. D. (2016). ATXN2-AS, a gene antisense to ATXN2, is associated with spinocerebellar ataxia type 2 and amyotrophic lateral sclerosis. *Annals of Neurology*, 80, 600–615. <https://doi.org/10.1002/ana.24761>
- Li, T., Xie, J., Shen, C., Cheng, D., Shi, Y., Wu, Z., Deng, X., Chen, H., Shen, B., Peng, C., Li, H., Zhan, Q., & Zhu, Z. (2016). Upregulation of long noncoding RNA ZEB1-AS1 promotes tumor metastasis and predicts poor prognosis in hepatocellular carcinoma. *Oncogene*, 35, 1575–1584. <https://doi.org/10.1038/onc.2015.223>
- Li, X., Yang, L., & Chen, L. L. (2018). The biogenesis, functions, and challenges of circular RNAs. *Molecular Cell*, 71(3), 428–442. <https://doi.org/10.1016/j.molcel.2018.06.034>
- Lipovich, L., Dachet, F., Cai, J., Bagla, S., Balan, K., Jia, H., & Loeb, J. A. (2012). Activity-dependent human brain coding/noncoding gene regulatory networks. *Genetics*, 192, 1133–1148. <https://doi.org/10.1534/genetics.112.145128>
- Liu, Y., Pattamatta, A., Zu, T., Reid, T., Bardhi, O., Borchelt, D. R., Yachnis, A. T., & Ranum, L. P. W. (2016). C9orf72 BAC mouse model with motor deficits and neurodegenerative features of ALS/FTD. *Neuron*, 90, 521–534. <https://doi.org/10.1016/j.neuron.2016.04.005>
- Lo Piccolo, L., & Yamaguchi, M. (2017). RNAi of arcRNA hsr ω affects sub-cellular localization of Drosophila FUS to drive neurodegeneration. *Experimental Neurology*, 292, 125–134. <https://doi.org/10.1016/j.expneurol.2017.03.011>
- Lorson, C. L., Hahnen, E., Androphy, E. J., & Wirth, B. (1999). A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. *Proceedings of the National Academy of Sciences*, 96, 6307–6311. <https://doi.org/10.1073/pnas.96.11.6307>
- Lu, Q. R., Sun, T., Zhu, Z., Ma, N., Garcia, M., Stiles, C. D., & Rowitch, D. H. (2002). Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. *Cell*, 109, 75–86. [https://doi.org/10.1016/S0092-8674\(02\)00678-5](https://doi.org/10.1016/S0092-8674(02)00678-5)
- Lunn, M., & Wang, C. (2016). Spinal muscular atrophy. *Neurology*, 86, 884–885. <https://doi.org/10.1212/WNL.0000000000002453>
- Ma, L., Bajic, V. B., & Zhang, Z. (2013). On the classification of long non-coding RNAs. *RNA Biology*, 10, 924–933. <https://doi.org/10.4161/rna.24604>
- Magaña, J. J., Velázquez-Pérez, L., & Cisneros, B. (2013). Spinocerebellar ataxia Type 2: Clinical presentation, molecular mechanisms, and therapeutic perspectives. *Molecular Neurobiology*, 47(1), 90–104. <https://doi.org/10.1007/s12035-012-8348-8>
- Majounie, E., Renton, A. E., Mok, K., Dopper, E. G. P., Waite, A., Rollinson, S., Chiò, A., Restagno, G., Nicolaou, N., Simon-Sanchez, J., van Swieten, J. C., Abramzon, Y., Johnson, J. O., Sendtner, M., Pampillet, R., Orrell, R. W., Mead, S., Sidle, K. C., Houlden, H., ... Logroscino, G. (2012). Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: A cross-sectional study. *The Lancet Neurology*, 11, 323–330. [https://doi.org/10.1016/S1474-4422\(12\)70043-1](https://doi.org/10.1016/S1474-4422(12)70043-1)
- Malecová, B., & Morris, K. V. (2010). Transcriptional gene silencing through epigenetic changes mediated by non-coding RNAs. *Current Opinion in Molecular Therapeutics*, 12(2), 214.
- Managadze, D., Lobkovsky, A. E., Wolf, Y. I., Shabalina, S. A., Rogozin, I. B., & Koonin, E. V. (2013). The vast, conserved mammalian lincRNome. *PLoS Computational Biology*, 9, e1002917. <https://doi.org/10.1371/journal.pcbi.1002917>
- Mao, Y. S., Sunwoo, H., Zhang, B., & Spector, D. L. (2011). Direct visualization of the co-transcriptional assembly of a nuclear body by noncoding RNAs. *Nature Cell Biology*, 13, 95–101. <https://doi.org/10.1038/ncb2140>
- Marchese, F. P., Raimondi, I., & Huarte, M. (2017). The multidimensional mechanisms of long noncoding RNA function. *Genome Biology*, 18, 1–13. <https://doi.org/10.1186/s13059-017-1348-2>
- Mehler, M. F., & Mattick, J. S. (2007). Noncoding RNAs and RNA editing in brain development, functional diversification, and neurological disease. *Physiological Reviews*, 87, 799–823. <https://doi.org/10.1152/physrev.00036.2006>
- Memczak, S., Jens, M., Elefsinioti, A., & Torti, F. (2013). Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature*, 495, 333–338. <https://doi.org/10.1038/nature11928>
- Meng, S., Zhou, H., Feng, Z., Xu, Z., Tang, Y., & Wu, M. (2019). Epigenetics in neurodevelopment: Emerging role of circular RNA. *Frontiers in Cellular Neuroscience*, 13, 1–12. <https://doi.org/10.3389/fncel.2019.00327>
- Mercer, T. R., Dinger, M. E., Sunkin, S. M., Mehler, M. F., & Mattick, J. S. (2008). Specific expression of long noncoding RNAs in the mouse brain. *Proceedings of the National Academy of Sciences*, 105(2), 716–721. <https://doi.org/10.1073/pnas.0706729105>
- Michalik, K. M., You, X., Manavski, Y., Doddaballapur, A., Zörnig, M., Braun, T., John, D., Ponomareva, Y., Chen, W., Uchida, S., Boon, R. A., & Dimmeler, S. (2014). Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth. *Circulation Research*, 114, 1389–1397. <https://doi.org/10.1161/CIRCRESAHA.114.303265>
- Michelhaugh, S. K., Lipovich, L., Blythe, J., Jia, H., Kapatoss, G., & Bannon, M. J. (2011). Mining Affymetrix microarray data for long non-coding RNAs: Altered expression in the nucleus accumbens of heroin abusers. *Journal of Neurochemistry*, 116, 459–466. <https://doi.org/10.1111/j.1471-4159.2010.07126.x>
- Militello, G., Hosen, M. R., Ponomareva, Y., Gellert, P., Weirick, T., John, D., Hindi, S. M., Mamchaoui, K., Mouly, V., Döring, C., Zhang, L., Nakamura, M., Kumar, A., Fukada, S. I., Dimmeler, S., & Uchida, S. (2018). A novel long non-coding RNA Myolinc regulates myogenesis through TDP-43 and Filip1. *Journal of Molecular Cell Biology*, 10, 102–117. <https://doi.org/10.1093/jmcb/mjy025>
- Mizielinska, S., Grönke, S., Niccoli, T., Ridler, C. E., Clayton, E. L., Devoy, A., Moens, T., Norona, F. E., Woollacott, I. O. C., Pietrzyk, J., Cleverley, K., Nicoll, A. J., Pickering-Brown, S., Dols, J., Cabecinha, M., Hendrich, O., Fratta, P., Fisher, E. M. C., Partridge, L., & Isaacs, A. M. (2014). C9orf72 repeat expansions cause neurodegeneration in Drosophila through arginine-rich proteins. *Science*, 345, 1192–1194. <https://doi.org/10.1126/science.1256800>
- Mizielinska, S., Lashley, T., Norona, F. E., Clayton, E. L., Ridler, C. E., Fratta, P., & Isaacs, A. M. (2013). C9orf72 frontotemporal lobar degeneration is characterised by frequent neuronal sense and antisense RNA foci. *Acta Neuropathologica*, 126, 845–857. <https://doi.org/10.1007/s00401-013-1200-z>
- Modarresi, F., Faghihi, M. A., Lopez-Toledano, M. A., Fatemi, R. P., Magistri, M., Brothers, S. P., Van Der Brug, M. P., & Wahlestedt, C. (2012). Inhibition of natural antisense transcripts in vivo results in gene-specific transcriptional upregulation. *Nature Biotechnology*, 30, 453–459. <https://doi.org/10.1038/nbt.2158>
- Moens, T. G., Mizielinska, S., Niccoli, T., Mitchell, J. S., Thoeng, A., Ridler, C. E., Grönke, S., Esser, J., Heslegrave, A., Zetterberg, H.,

- Partridge, L., & Isaacs, A. M. (2018). Sense and antisense RNA are not toxic in *Drosophila* models of C9orf72-associated ALS/FTD. *Acta Neuropathologica*, 135, 445–457. <https://doi.org/10.1007/s00401-017-1798-3>
- Moens, T. G., Partridge, L., & Isaacs, A. M. (2017). Genetic models of C9orf72: What is toxic? *Current Opinion in Genetics and Development*, 44, 92–101. <https://doi.org/10.1016/j.gde.2017.01.006>
- Monani, U. R., Lorson, C. L., Parsons, D. W., Prior, T. W., Androphy, E. J., Burghes, A. H. M., & McPherson, J. D. (1999). A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. *Human Molecular Genetics*, 8, 1177–1183. <https://doi.org/10.1093/hmg/8.7.1177>
- Monia, B. P., Johnston, J. F., Ecker, D. J., Zounes, M. A., Lima, W. F., & Freier, S. M. (1992). Selective inhibition of mutant Ha-ras mRNA expression by antisense oligonucleotides. *Journal of Biological Chemistry*, 267, 19954–19962.
- Mori, K., Arzberger, T., Grässer, F. A., Gijssels, I., May, S., Rentzsch, K., Weng, S. M., Schludi, M. H., Van Der Zee, J., Cruts, M., Van Broeckhoven, C., Kremmer, E., Kretzschmar, H. A., Haass, C., & Edbauer, D. (2013). Bidirectional transcripts of the expanded C9orf72 hexanucleotide repeat are translated into aggregating dipeptide repeat proteins. *Acta Neuropathologica*, 126, 881–893. <https://doi.org/10.1007/s00401-013-1189-3>
- Mori, K., Weng, S. M., Arzberger, T., May, S., Rentzsch, K., Kremmer, E., Schmid, B., Kretzschmar, H. A., Cruts, M., Van Broeckhoven, C., Haass, C., & Edbauer, D. (2013). The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTL/ALS. *Science*, 339, 1335–1338. <https://doi.org/10.1126/science.1232927>
- Naganuma, T., Nakagawa, S., Tanigawa, A., Sasaki, Y. F., Goshima, N., & Hirose, T. (2012). Alternative 3'-end processing of long noncoding RNA initiates construction of nuclear paraspeckles. *EMBO Journal*, 31, 4020–4034. <https://doi.org/10.1038/emboj.2012.251>
- Nishimoto, Y., Nakagawa, S., Hirose, T., Okano, H. J., Takao, M., Shibata, S., Suyama, S., Kuwako, K. I., Imai, T., Murayama, S., Suzuki, N., & Okano, H. (2013). The long non-coding RNA nuclear-enriched abundant transcript 1–2 induces paraspeckle formation in the motor neuron during the early phase of amyotrophic lateral sclerosis. *Molecular Brain*, 6, 1–18. <https://doi.org/10.1186/1756-6606-6-31>
- Nishimura, A. L., Mitne-Neto, M., Silva, H. C. A., Richieri-Costa, A., Middleton, S., Cascio, D., Kok, F., Oliveira, J. R. M., Gillingwater, T., Webb, J., Skehel, P., & Zatz, M. (2004). A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *American Journal of Human Genetics*, 75, 822–831. <https://doi.org/10.1086/425287>
- Noh, J. H., Kim, K. M., McClusky, W. G., Abdelmohsen, K., & Gorospe, M. (2018). Cytoplasmic functions of long noncoding RNAs. *Wiley Interdisciplinary Reviews: RNA*, 9, e1471. <https://doi.org/10.1002/wrna.1471>
- O'Rourke, J. G., Bogdanik, L., Muhammad, A. K. M. G., Gendron, T. F., Kim, K. J., Austin, A., Cady, J., Liu, E. Y., Zarrow, J., Grant, S., Ho, R., Bell, S., Carmona, S., Simpkinson, M., Lall, D., Wu, K., Daugherty, L., Dickson, D. W., Harms, M. B., ... Baloh, R. H. (2015). C9orf72 BAC transgenic mice display typical pathologic features of ALS/FTD. *Neuron*, 88, 892–901. <https://doi.org/10.1016/j.neuron.2015.10.027>
- Ogata, T., & Kagami, M. (2016). Kagami-Ogata syndrome: A clinically recognizable upd(14)pat and related disorder affecting the chromosome 14q32.2 imprinted region. *Journal of Human Genetics*, 61(2), 87–94. <https://doi.org/10.1038/jhg.2015.113>
- Ottesen, E. W., Luo, D., Seo, J., Singh, N. N., & Singh, R. N. (2019). Human survival motor neuron genes generate a vast repertoire of circular RNAs. *Nucleic Acids Research*, 47, 2884–2905. <https://doi.org/10.1093/nar/gkz034>
- Palazzo, A. F., & Lee, E. S. (2015). Non-coding RNA: What is functional and what is junk? *Front. Genet.*, 5, 2. <https://doi.org/10.3389/fgene.2015.00002>
- Papaiannou, D., Petri, A., Dovey, O. M., Terreri, S., Wang, E., Collins, F. A., Woodward, L. A., Walker, A. E., Nicolet, D., Pepe, F., Kumchala, P., Bill, M., Walker, C. J., Karunasiri, M., Mrózek, K., Gardner, M. L., Camilotto, V., Zitzer, N., Cooper, J. L., ... Garzon, R. (2019). The long non-coding RNA HOXB-AS3 regulates ribosomal RNA transcription in NPM1-mutated acute myeloid leukemia. *Nature Communications*, 10, 1–15. <https://doi.org/10.1038/s41467-019-13259-2>
- Park, H. C., Mehta, A., Richardson, J. S., & Appel, B. (2002). olig2 is required for zebrafish primary motor neuron and oligodendrocyte development. *Developmental Biology*, 248, 356–368. <https://doi.org/10.1006/dbio.2002.0738>
- Pereira Fernandes, D., Bitar, M., Jacobs, F., & Barry, G. (2018). Long non-coding RNAs in neuronal aging. *Non-Coding RNA*, 4, 12. <https://doi.org/10.3390/ncrna4020012>
- Peters, O. M., Cabrera, G. T., Tran, H., Gendron, T. F., McKeon, J. E., Metterville, J., Weiss, A., Wightman, N., Salameh, J., Kim, J., Sun, H., Boylan, K. B., Dickson, D., Kennedy, Z., Lin, Z., Zhang, Y. J., Daugherty, L., Jung, C., Gao, F. B., ... Brown, R. H. (2015). Human C9ORF72 hexanucleotide expansion reproduces RNA foci and dipeptide repeat proteins but not neurodegeneration in BAC transgenic mice. *Neuron*, 88, 902–909. <https://doi.org/10.1016/j.neuron.2015.11.018>
- Polikepahad, S., & Corry, D. B. (2013). Profiling of T helper cell-derived small RNAs reveals unique antisense transcripts and differential association of miRNAs with argonaute proteins 1 and 2. *Nucleic Acids Research*, 41, 1164–1177. <https://doi.org/10.1093/nar/gks1098>
- Ponjavic, J., Oliver, P. L., Lunter, G., & Ponting, C. P. (2009). Genomic and transcriptional co-localization of protein-coding and long non-coding RNA pairs in the developing brain. *PLoS Genetics*, 5, e1000617. <https://doi.org/10.1371/journal.pgen.1000617>
- Quan, Z., Zheng, D., & Qing, H. (2017). Regulatory roles of long non-coding RNAs in the central nervous system and associated neurodegenerative diseases. *Frontiers in Cellular Neuroscience*, 11, 175. <https://doi.org/10.3389/fncel.2017.00175>
- Quinn, J. J., & Chang, H. Y. (2016). Unique features of long non-coding RNA biogenesis and function. *Nature Reviews Genetics*, 17, 47–62. <https://doi.org/10.1038/nrg.2015.10>
- Qureshi, I. A., & Mehler, M. F. (2012). Emerging roles of non-coding RNAs in brain evolution, development, plasticity and disease. *Nature Reviews Neuroscience*, 13, 528–541. <https://doi.org/10.1038/nnr3234>
- Rademakers, R. (2012). C9orf72 repeat expansions in patients with ALS and FTD. *The Lancet Neurology*, 11, 297–298. [https://doi.org/10.1016/S1474-4422\(12\)70046-7](https://doi.org/10.1016/S1474-4422(12)70046-7)
- Ransohoff, J. D., Wei, Y., & Khavari, P. A. (2018). Functions and unique features of long intergenic non-coding RNA. *Nature Reviews Molecular Cell Biology*, 19(3), 143–157. <https://doi.org/10.1038/nrm.2017.104>
- Rashid, F., Shah, A., & Shan, G. (2016). Long non-coding RNAs in the cytoplasm. *Genomics, Proteomics and Bioinformatics*, 14, 73–80. <https://doi.org/10.1016/j.gpb.2016.03.005>
- Ravanelli, A. M., & Appel, B. (2015). Motor neurons and oligodendrocytes arise from distinct cell lineages by progenitor recruitment. *Genes and Development*, 29, 2504–2515. <https://doi.org/10.1101/gad.271312.115>
- Ravasi, T., Suzuki, H., Pang, K. C., Katayama, S., Furuno, M., Okunishi, R., Fukuda, S., Ru, K., Frith, M. C., Gongora, M. M., Grimmond, S. M., Hume, D. A., Hayashizaki, Y., & Mattick, J. S. (2006). Experimental validation of the regulated expression of large numbers of non-coding RNAs from the mouse genome. *Genome Research*, 16, 11–19. <https://doi.org/10.1101/gr.4200206>
- Ray, M. K., Wiskow, O., King, M. J., Ismail, N., Ergun, A., Wang, Y., Plys, A. J., Davis, C. P., Kathrein, K., Sadreyev, R., Borowsky, M. L., Eggan, K., Zon, L., Galloway, J. L., & Kingston, R. E. (2016). CAT7 and cat7l long non-coding RNAs tune polycomb repressive complex 1 function during human and zebrafish development. *Journal of*



- Biological Chemistry*, 291, 19558–19572. <https://doi.org/10.1074/jbc.M116.730853>
- Reddy, K., Zamiri, B., Stanley, S. Y. R., Macgregor, R. B., & Pearson, C. E. (2013). The disease-associated r(GGGGCC)_n repeat from the C9orf72 gene forms tract length-dependent uni- and multimolecular RNA G-quadruplex structures. *Journal of Biological Chemistry*, 288, 9860–9866. <https://doi.org/10.1074/jbc.C113.452532>
- Renton, A. E., Chiò, A., & Traynor, B. J. (2014). State of play in amyotrophic lateral sclerosis genetics. *Nature Neuroscience*, 17, 17–23. <https://doi.org/10.1038/nn.3584>
- Renton, A. E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J. R., Schymick, J. C., Laaksovirta, H., van Swieten, J. C., Myllykangas, L., Kalimo, H., Paetau, A., Abramzon, Y., Remes, A. M., Kaganovich, A., Scholz, S. W., Duckworth, J., Ding, J., Harmer, D. W., ... Traynor, B. J. (2011). A hexanucleotide repeat expansion in C9orf72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron*, 72, 257–268. <https://doi.org/10.1016/j.neuron.2011.09.010>
- Rinn, J. L., & Chang, H. Y. (2012). Genome regulation by long noncoding RNAs. *Annual Review of Biochemistry*, 81, 145–166. <https://doi.org/10.1146/annurev-biochem-051410-092902>
- Rinn, J. L., Kertesz, M., Wang, J. K., Squazzo, S. L., Xu, X., Bruggmann, S. A., Goodnough, L. H., Helms, J. A., Farnham, P. J., Segal, E., & Chang, H. Y. (2007). Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell*, <https://doi.org/10.1016/j.cell.2007.05.022>
- Rizvi, A., Camara, P. G., Kandrór, E. K., Roberts, T. J., Schieren, I., Maniatis, T., & Rabadan, R. (2017). Single-cell topological RNA-seq analysis reveals insights into cellular differentiation and development. *Nature Biotechnology*, 35, 551–560. <https://doi.org/10.1038/nbt.3854>
- Robberecht, W., & Philips, T. (2013). The changing scene of amyotrophic lateral sclerosis. *Nature Reviews Neuroscience*, 14, 248–264. <https://doi.org/10.1038/nrn3430>
- Rybak-Wolf, A., Stottmeister, C., Glažar, P., Jens, M., Pino, N., Hanan, M., Behm, M., Bartok, O., Ashwal-Fluss, R., Herzog, M., Schreyer, L., Papavasileiou, P., Ivanov, A., Öhman, M., Refojo, D., Kadener, S., & Rajewsky, N. (2015). Circular RNAs in the mammalian brain are highly abundant, conserved, and dynamically expressed. *Molecular Cell*, 58, 870–885. <https://doi.org/10.1016/j.molcel.2015.03.027>
- Saberi, S., Stauffer, J. E., Jiang, J., Garcia, S. D., Taylor, A. E., Schulte, D., Ohkubo, T., Schloffman, C. L., Maldonado, M., Baughn, M., Rodriguez, M. J., Pizzo, D., Cleveland, D., & Ravits, J. (2018). Sense-encoded poly-GR dipeptide repeat proteins correlate to neurodegeneration and uniquely co-localize with TDP-43 in dendrites of repeat-expanded C9orf72 amyotrophic lateral sclerosis. *Acta Neuropathologica*, 135, 459–474. <https://doi.org/10.1007/s00401-017-1793-8>
- Salzman, J. (2016). Circular RNA expression: Its potential regulation and function. *Trends in Genetics*, 32, 309–316. <https://doi.org/10.1016/j.tig.2016.03.002>
- Salzman, J., Gawad, C., Wang, P. L., Lacayo, N., & Brown, P. O. (2012). Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS One*, 7, e30733. <https://doi.org/10.1371/journal.pone.0030733>
- Sareen, D., O'Rourke, J. G., Meera, P., Muhammad, A. K. M. G., Grant, S., Simpkinson, M., Bell, S., Carmona, S., Ornelas, L., Sahabian, A., Gendron, T., Petrucelli, L., Baughn, M., Ravits, J., Harms, M. B., Rigo, F., Bennett, C. F., Otis, T. S., Svendsen, C. N., & Baloh, R. H. (2013). Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a C9orf72 repeat expansion. *Science Translational Medicine*, 5, 208ra149. <https://doi.org/10.1126/scitranslmed.3007529>
- Satterfield, T. F., & Pallanck, L. J. (2006). Ataxin-2 and its Drosophila homolog, ATX2, physically assemble with polyribosomes. *Human Molecular Genetics*, 15, 2523–2532. <https://doi.org/10.1093/hmg/ddl173>
- Schoch, K. M., & Miller, T. M. (2017). Antisense oligonucleotides: Translation from mouse models to human neurodegenerative diseases. *Neuron*, 94, 1056–1070. <https://doi.org/10.1016/j.neuron.2017.04.010>
- Scoles, D. R., Minikel, E. V., & Pulst, S. M. (2019). Antisense oligonucleotides. *Neurology Genetics*, 5, e323. <https://doi.org/10.1212/NXG.0000000000000323>
- Scoles, D. R., & Pulst, S. M. (2018). Spinocerebellar ataxia type 2. *Advances in experimental medicine and biology* (pp. 175–195). Springer.
- Sekar, S., & Liang, W. S. (2019). Circular RNA expression and function in the brain. *Non-coding RNA Research*, 4, 23–29. <https://doi.org/10.1016/j.ncrna.2019.01.001>
- Sewell, K. L., Geary, R. S., Baker, B. F., Glover, J. M., Mant, T. G. K., Yu, R. Z., Tami, J. A., & Dorr, F. A. (2002). Phase I trial of ISIS 104838, a 2'-methoxyethyl modified antisense oligonucleotide targeting tumor necrosis factor- α . *Journal of Pharmacology and Experimental Therapeutics*, 303, 1334–1343. <https://doi.org/10.1124/jpet.102.036749>
- Shelkovnikova, T. A., Kukharsky, M. S., An, H., Dimasi, P., Alexeeva, S., Shabir, O., Heath, P. R., & Buchman, V. L. (2018). Protective paraspeckle hyper-assembly downstream of TDP-43 loss of function in amyotrophic lateral sclerosis. *Molecular Neurodegeneration*, 13, 1–17. <https://doi.org/10.1186/s13024-018-0263-7>
- Shelkovnikova, T. A., Robinson, H. K., Troakes, C., Ninkina, N., & Buchman, V. L. (2014). Compromised paraspeckle formation as a pathogenic factor in FUSopathies. *Human Molecular Genetics*, 23, 2298–2312. <https://doi.org/10.1093/hmg/ddt622>
- Shi, C., Zhang, L., & Qin, C. (2017). Long non-coding RNAs in brain development, synaptic biology, and Alzheimer's disease. *Brain Research Bulletin*, 132, 160–169. <https://doi.org/10.1016/j.brainresbu.2017.03.010>
- Simchovitz, A., Hanan, M., Niederhoffer, N., Madrer, N., Yayon, N., Bennett, E. R., Greenberg, D. S., Kadener, S., & Soreq, H. (2019). NEAT1 is overexpressed in Parkinson's disease substantia nigra and confers drug-inducible neuroprotection from oxidative stress. *The FASEB Journal*, 33, 11223–11234. <https://doi.org/10.1096/fj.20190830R>
- Simone, R., Balendra, R., Moens, T. G., Preza, E., Wilson, K. M., Heslegrave, A., Woodling, N. S., Niccoli, T., Gilbert-Jaramillo, J., Abdelkarim, S., Clayton, E. L., Clarke, M., Konrad, M., Nicoll, A. J., Mitchell, J. S., Calvo, A., Chio, A., Houlden, H., Polke, J. M., ... Isaacs, A. M. (2018). G-quadruplex-binding small molecules ameliorate C9orf72 FTD/ALS pathology in vitro and in vivo. *EMBO Molecular Medicine*, 10, 22–31. <https://doi.org/10.15252/emmm.201707850>
- Sone, M., Hayashi, T., Tarui, H., Agata, K., Takeichi, M., & Nakagawa, S. (2007). The mRNA-like noncoding RNA Gomafu constitutes a novel nuclear domain in a subset of neurons. *Journal of Cell Science*, 120, 2498–2506. <https://doi.org/10.1242/jcs.009357>
- Sun, Q., Hao, Q., & Prasanth, K. V. (2018). Nuclear long noncoding RNAs: Key regulators of gene expression. *Trends in Genetics*, 34(2), 142–157. <https://doi.org/10.1016/j.tig.2017.11.005>
- Sunwoo, H., Dinger, M. E., Wilusz, J. E., Amaral, P. P., Mattick, J. S., & Spector, D. L. (2009). Men ϵ/β nuclear-retained non-coding RNAs are up-regulated upon muscle differentiation and are essential components of paraspeckles. *Genome Research*, 19, 347–359. <https://doi.org/10.1101/gr.087775.108>
- Sunwoo, J. S., Lee, S. T., Im, W., Lee, M., Byun, J. I., Jung, K. H., Park, K. I., Jung, K. Y., Lee, S. K., Chu, K., & Kim, M. (2017). Altered expression of the long noncoding RNA NEAT1 in Huntington's disease. *Molecular Neurobiology*, 54, 1577–1586. <https://doi.org/10.1007/s12035-016-9928-9>
- Suzuki, H., Shibagaki, Y., Hattori, S., & Matsuoka, M. (2019). C9-ALS/FTD-linked proline-arginine dipeptide repeat protein associates with paraspeckle components and increases paraspeckle formation. *Cell Death and Disease*, 10, 746. <https://doi.org/10.1038/s41419-019-1983-5>
- Swinnen, B., Bento-Abreu, A., Gendron, T. F., Boeynaems, S., Bogaert, E., Nuyts, R., Timmers, M., Scheveneels, W., Hersmus, N., Wang, J.,



- Mizielinska, S., Isaacs, A. M., Petrucelli, L., Lemmens, R., Van Damme, P., Van Den Bosch, L., & Robberecht, W. (2018). A zebrafish model for C9orf72 ALS reveals RNA toxicity as a pathogenic mechanism. *Acta Neuropathologica*, 135, 427–443. <https://doi.org/10.1007/s00401-017-1796-5>
- Swinnen, B., Robberecht, W., & Van Den Bosch, L. (2020). RNA toxicity in non-coding repeat expansion disorders. *EMBO Journal*, 39, 1–23. <https://doi.org/10.15252/embj.2018101112>
- Tao, Z., Wang, H., Xia, Q., Li, K. E., Li, K., Jiang, X., Xu, G., Wang, G., & Ying, Z. (2015). Nucleolar stress and impaired stress granule formation contribute to C9orf72 RAN translation-induced cytotoxicity. *Human Molecular Genetics*, 24, 2426–2441. <https://doi.org/10.1093/hmg/ddv005>
- Tollervey, J. R., Curk, T., Rogelj, B., Briese, M., Cereda, M., Kayikci, M., König, J., Hortobágyi, T., Nishimura, A. L., Župunski, V., Patani, R., Chandran, S., Rot, G., Zupan, B., Shaw, C. E., & Ule, J. (2011). Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. *Nature Neuroscience*, 14, 452–458. <https://doi.org/10.1038/nn.2778>
- Tung, Y. T., Peng, K. C., Chen, Y. C., Yen, Y. P., Chang, M., Thams, S., & Chen, J. A. (2019). Mir-17~92 Confers motor neuron subtype differential resistance to ALS-associated degeneration. *Cell Stem Cell*, 25, 193–209.e7. <https://doi.org/10.1016/j.stem.2019.04.016>
- Uliutsky, I., & Bartel, D. P. (2013). XlincRNAs: Genomics, evolution, and mechanisms. *Cell*, <https://doi.org/10.1016/j.cell.2013.06.020>
- Uliutsky, I., Shkumatava, A., Jan, C. H., Sive, H., & Bartel, D. P. (2011). Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution. *Cell*, 147, 1537–1550. <https://doi.org/10.1016/j.cell.2011.11.055>
- Van Arensbergen, J., García-Hurtado, J., Moran, I., Maestro, M. A., Xu, X., Van De Castele, M., Skoudy, A. L., Palassini, M., Heimberg, H., & Ferrer, J. (2010). Derepression of polycomb targets during pancreatic organogenesis allows insulin-producing beta-cells to adopt a neural gene activity program. *Genome Research*, 20, 722–732. <https://doi.org/10.1101/gr.101709.109>
- Van den Heuvel, D. M. A., Harschnitz, O., van den Berg, L. H., & Pasterkamp, R. J. (2014). Taking a risk: A therapeutic focus on ataxin-2 in amyotrophic lateral sclerosis? *Trends Mol. Med.*, 20, 25–35. <https://doi.org/10.1016/j.molmed.2013.09.001>
- van Es, M. A., Hardiman, O., Chio, A., Al-Chalabi, A., Pasterkamp, R. J., Veldink, J. H., & van den Berg, L. H. (2017). Amyotrophic lateral sclerosis. *Lancet*, 390, 2084–2098. [https://doi.org/10.1016/S0140-6736\(17\)31287-4](https://doi.org/10.1016/S0140-6736(17)31287-4)
- van Rossum, D., Verheijen, B. M., & Pasterkamp, R. J. (2016). Circular RNAs: Novel regulators of neuronal development. *Frontiers in Molecular Neuroscience*, 9, 1–7. <https://doi.org/10.3389/fnmol.2016.00074>
- Venø, M. T., Hansen, T. B., Venø, S. T., Clausen, B. H., Grebing, M., Finsen, B., Holm, I. E., & Kjems, J. (2015). Spatio-temporal regulation of circular RNA expression during porcine embryonic brain development. *Genome Biology*, 16, 245. <https://doi.org/10.1186/s13059-015-0801-3>
- Verheijen, B. M., & Pasterkamp, R. J. (2017). Commentary: FUS affects circular RNA expression in murine embryonic stem cell-derived motor neurons. *Frontiers in Molecular Neuroscience*, 10, 412. <https://doi.org/10.3389/fnmol.2017.00412>
- Villar-Menéndez, I., Porta, S., Buira, S. P., Pereira-Veiga, T., Díaz-Sánchez, S., Albasanz, J. L., Ferrer, I., Martín, M., & Barrachina, M. (2014). Increased striatal adenosine A2A receptor levels is an early event in Parkinson's disease-related pathology and it is potentially regulated by miR-34b. *Neurobiology of Diseases*, 69, 206–214. <https://doi.org/10.1016/j.nbd.2014.05.030>
- Vollmer, J., Jepsen, J. S., Uhlmann, E., Schetter, C., Jurk, M., Wader, T., Wüllner, M., & Krieg, A. M. (2004). Modulation of CpG oligodeoxynucleotide-mediated immune stimulation by locked nucleic acid (LNA). *Oligonucleotides*, 14, 23–31. <https://doi.org/10.1089/154545704322988021>
- Wang, F., Liang, R., Soibam, B., Yang, J., & Liu, Y. (2019). Coregulatory long non-coding RNA and protein-coding genes in serum starved cells. *Biochimica Et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1862, 84–95. <https://doi.org/10.1016/j.bbagr.2018.11.004>
- Wang, K. C., & Chang, H. Y. (2011). Molecular mechanisms of long non-coding RNAs. *Molecular Cell*, 43, 904–914. <https://doi.org/10.1016/j.molcel.2011.08.018>
- Wang, K. C., Yang, Y. W., Liu, B., Sanyal, A., Corces-Zimmerman, R., Chen, Y., Lajoie, B. R., Protacio, A., Flynn, R. A., Gupta, R. A., Wysocka, J., Lei, M., Dekker, J., Helms, J. A., & Chang, H. Y. (2011). A long non-coding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature*, 472, 120–126. <https://doi.org/10.1038/nature09819>
- Wang, S. M., Liu, G. Q., Xian, H. B., Si, J. L., Qi, S. X., & Yu, Y. P. (2019). LncRNA NEAT1 alleviates sepsis-induced myocardial injury by regulating the TLR2/NF- κ B signaling pathway. *European Review for Medical and Pharmacological Sciences*, 23, 4898–4907. https://doi.org/10.26355/eurrev_201906_18078
- Wang, X., Goodrich, K. J., Conlon, E. G., Gao, J., Erbse, A. H., Manley, J. L., & Cech, T. R. (2019). C9orf72 and triplet repeat disorder RNAs: G-quadruplex formation, binding to PRC2 and implications for disease mechanisms. *RNA*, 25, 935–947. <https://doi.org/10.1261/rna.071191.119>
- Wang, X.-M., Li, X.-M., Song, N., Zhai, H., Gao, X.-M., & Yang, Y.-N. (2019). Long non-coding RNAs H19, MALAT1 and MIAT as potential novel biomarkers for diagnosis of acute myocardial infarction. *Biomedicine and Pharmacotherapy*, 118, 109208. <https://doi.org/10.1016/j.biopha.2019.109208>
- Wang, Z., Xu, P., Chen, B., Zhang, Z., Zhang, C., Zhan, Q., Huang, S., Xia, Z. A., & Peng, W. (2018). Identifying circRNA-associated-ceRNA networks in the hippocampus of A β 1-42-induced Alzheimer's disease-like rats using microarray analysis. *Aging (Albany, NY)*, 10, 775–788. <https://doi.org/10.18632/aging.101427>
- Wen, X., Tan, W., Westergard, T., Krishnamurthy, K., Markandaiah, S. S., Shi, Y., Lin, S., Shneider, N. A., Monaghan, J., Pandey, U. B., Pasinelli, P., Ichida, J. K., & Trotti, D. (2014). Antisense proline-arginine RAN dipeptides linked to C9ORF72-ALS/FTD form toxic nuclear aggregates that initiate invitro and invivo neuronal death. *Neuron*, 84, 1213–1225. <https://doi.org/10.1016/j.neuron.2014.12.010>
- Werner, A., Cockell, S., Falconer, J., Carlile, M., Alnumeir, S., & Robinson, J. (2014). Contribution of natural antisense transcription to an endogenous siRNA signature in human cells. *BMC Genomics*, 15, 19. <https://doi.org/10.1186/1471-2164-15-19>
- Westholm, J. O., Miura, P., Olson, S., Shenker, S., Joseph, B., Sanfilippo, P., Celniker, S. E., Graveley, B. R., & Lai, E. C. (2014). Genome-wide analysis of drosophila circular RNAs reveals their structural and sequence properties and age-dependent neural accumulation. *Cell Reports*, 9, 1966–1980. <https://doi.org/10.1016/j.celrep.2014.10.062>
- Wight, M., & Werner, A. (2013). The functions of natural antisense transcripts. *Essays in Biochemistry*, 54, 91–101. <https://doi.org/10.1042/BSE0540091>
- Wijesekera, L. C., & Leigh, P. N. (2009). Amyotrophic lateral sclerosis. *Orphanet Journal of Rare Diseases*, 4, 3. <https://doi.org/10.1186/1750-1172-4-3>
- Woo, C. J., Maier, V. K., Davey, R., Brennan, J., Li, G., Brothers, J., Schwartz, B., Gordo, S., Kasper, A., Okamoto, T. R., Johansson, H. E., Mandefro, B., Sareen, D., Bialek, P., Chau, B. N., Bhat, B., Bullough, D., & Barsoum, J. (2017). Gene activation of SMN by selective disruption of lncRNA-mediated recruitment of PRC2 for the treatment of spinal muscular atrophy. *Proceedings of the National Academy of Sciences*, 114, E1509–E1518. <https://doi.org/10.1073/pnas.1616521114>
- Wu, L., Liu, C., & Zhang, Z. (2020). Knockdown of lncRNA MIAT inhibits proliferation and cisplatin resistance in non-small cell lung cancer



- cells by increasing miR-184 expression. *Oncology Letters*, 19, 533–541. <https://doi.org/10.3892/ol.2019.11084>
- Xiao, S., Sanelli, T., Dib, S., Sheps, D., Findlater, J., Bilbao, J., Keith, J., Zinman, L., Rogaeva, E., & Robertson, J. (2011). RNA targets of TDP-43 identified by UV-CLIP are deregulated in ALS. *Molecular and Cellular Neurosciences*, 47, 167–180. <https://doi.org/10.1016/j.mcn.2011.02.013>
- Xu, Z., Poidevin, M., Li, X., Li, Y., Shu, L., Nelson, D. L., Li, H., Hales, C. M., Gearing, M., Wingo, T. S., & Jin, P. (2013). Expanded GGGGCC repeat RNA associated with amyotrophic lateral sclerosis and frontotemporal dementia causes neurodegeneration. *Proceedings of the National Academy of Sciences*, 110, 7778–7783. <https://doi.org/10.1073/pnas.1219643110>
- Yamazaki, T., & Hirose, T. (2015). The building process of the functional paraspeckle with long non-coding RNAs. *Frontiers in Bioscience*, 7, 1–47. <https://doi.org/10.2741/715>
- Yamazaki, T., Souquere, S., Chujo, T., Kobelke, S., Chong, Y. S., Fox, A. H., Bond, C. S., Nakagawa, S., Pierron, G., & Hirose, T. (2018). Functional domains of NEAT1 architectural lncRNA induce paraspeckle assembly through phase separation. *Molecular Cell*, 70, 1038–1053.e7. <https://doi.org/10.1016/j.molcel.2018.05.019>
- Yan, L., Yang, M., Guo, H., Yang, L., Wu, J., Li, R., Liu, P., Lian, Y., Zheng, X., Yan, J., Huang, J., Li, M., Wu, X., Wen, L., Lao, K., Li, R., Qiao, J., & Tang, F. (2013). Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. *Nature Structural and Molecular Biology*, 20, 1131–1139. <https://doi.org/10.1038/nsmb.2660>
- Yen, Y.-P., Hsieh, W.-F., Tsai, Y.-Y., Lu, Y.-L., Liao, E. S., Hsu, H.-C., Chen, Y.-C., Liu, T.-C., Chang, M., Li, J., Lin, S.-P., Hung, J.-H., & Chen, J.-A. (2018). Dlk1-Dio3 locus-derived lncRNAs perpetuate postmitotic motor neuron cell fate and subtype identity. *Elife*, 7, 1–35. <https://doi.org/10.7554/eLife.38080>
- Yokoshi, M., Li, Q., Yamamoto, M., Okada, H., Suzuki, Y., & Kawahara, Y. (2014). Direct binding of Ataxin-2 to distinct elements in 3' UTRs promotes mRNA stability and protein expression. *Molecular Cell*, 55, 186–198. <https://doi.org/10.1016/j.molcel.2014.05.022>
- You, X., Vlatkovic, I., Babic, A., Will, T., Epstein, I., Tushev, G., Akbalik, G., Wang, M., Glock, C., Quedenau, C., Wang, X., Hou, J., Liu, H., Sun, W., Sambandan, S., Chen, T., Schuman, E. M., & Chen, W. (2015). Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. *Nature Neuroscience*, 18(4), 603–610. <https://doi.org/10.1038/nn.3975>
- Yu, W., Gius, D., Onyango, P., Muldoon-Jacobs, K., Karp, J., Feinberg, A. P., & Cui, H. (2008). Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA. *Nature*, 451, 202–206. <https://doi.org/10.1038/nature06468>
- Yuan, P., Cao, W., Zang, Q., Li, G., Guo, X., & Fan, J. (2016). The HIF-2 α -MALAT1-miR-216b axis regulates multi-drug resistance of hepatocellular carcinoma cells via modulating autophagy. *Biochemical and Biophysical Research Communications*, 478, 1067–1073. <https://doi.org/10.1016/j.bbrc.2016.08.065>
- Yunusov, D., Anderson, L., DaSilva, L. F., Wysocka, J., Ezashi, T., Roberts, R. M., & Verjovski-Almeida, S. (2016). HIPSTR and thousands of lncRNAs are heterogeneously expressed in human embryos, primordial germ cells and stable cell lines. *Scientific Reports*, 6, 32753. <https://doi.org/10.1038/srep32753>
- Zamiri, B., Reddy, K., Macgregor, R. B., & Pearson, C. E. (2014). TMPyP4 porphyrin distorts RNA G-quadruplex structures of the disease-associated r(GGGGCC) $_n$ repeat of the C9orf72 gene and blocks interaction of RNA-binding proteins. *Journal of Biological Chemistry*, 289, 4653–4659. <https://doi.org/10.1074/jbc.C113.502336>
- Zhang, H., Xing, L., Rossoll, W., Wichterle, H., Singer, R. H., & Bassell, G. J. (2006). Multiprotein complexes of the survival of motor neuron protein SMN with Gemins traffic to neuronal processes and growth cones of motor neurons. *Journal of Neuroscience*, 26, 8622–8632. <https://doi.org/10.1523/JNEUROSCI.3967-05.2006>
- Zhang, K., Donnelly, C. J., Haeusler, A. R., Grima, J. C., Machamer, J. B., Steinwald, P., Daley, E. L., Miller, S. J., Cunningham, K. M., Vidensky, S., Gupta, S., Thomas, M. A., Hong, I., Chiu, S. L., Haganir, R. L., Ostrow, L. W., Matunis, M. J., Wang, J., Sattler, R., ... Rothstein, J. D. (2015). The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. *Nature*, 525, 56–61. <https://doi.org/10.1038/nature14973>
- Zhang, Q., Chen, C.-Y., Yedavalli, V. S. R. K., & Jeang, K.-T. (2013). NEAT1 long noncoding RNA and paraspeckle bodies modulate HIV-1 posttranscriptional expression. *Mbio*, 4, <https://doi.org/10.1128/mBio.00596-12>
- Zhang, Q.-S., Wang, Z.-H., Zhang, J.-L., Duan, Y.-L., Li, G.-F., & Zheng, D.-L. (2016). Beta-asarone protects against MPTP-induced Parkinson's disease via regulating long non-coding RNA MALAT1 and inhibiting α -synuclein protein expression. *Biomedicine and Pharmacotherapy*, 83, 153–159. <https://doi.org/10.1016/j.biopha.2016.06.017>
- Zhang, X., Gejman, R., Mahta, A., Zhong, Y., Rice, K. A., Zhou, Y., Cheunsuchon, P., Louis, D. N., & Klibanski, A. (2010). Maternally expressed gene 3, an imprinted noncoding RNA gene, is associated with meningioma pathogenesis and progression. *Cancer Research*, 70, 2350–2358. <https://doi.org/10.1158/0008-5472.CAN-09-3885>
- Zhang, X., Hamblin, M. H., & Yin, K. J. (2017). The long noncoding RNA Malat 1: Its physiological and pathophysiological functions. *RNA Biology*, <https://doi.org/10.1080/15476286.2017.1358347>
- Zhang, X., Xie, K., Zhou, H., Wu, Y., Li, C., Liu, Y., Liu, Z., Xu, Q., Liu, S., Xiao, D., & Tao, Y. (2020). Role of non-coding RNAs and RNA modifiers in cancer therapy resistance. *Molecular Cancer*, 19(1), <https://doi.org/10.1186/s12943-020-01171-z>
- Zhao, J., Dahle, D., Zhou, Y., Zhang, X., & Klibanski, A. (2005). Hypermethylation of the promoter region is associated with the loss of MEG3 gene expression in human pituitary tumors. *Journal of Clinical Endocrinology and Metabolism*, 90, 2179–2186. <https://doi.org/10.1210/jc.2004-1848>
- Zhu, J., Zhang, R., Yang, D., Li, J., Yan, X., Jin, K., Li, W., Liu, X., Zhao, J., Shang, W., & Yu, T. (2018). Knockdown of long non-coding RNA XIST inhibited doxorubicin resistance in colorectal cancer by upregulation of miR-124 and downregulation of SGK1. *Cellular Physiology and Biochemistry*, 51, 113–128. <https://doi.org/10.1159/000495168>
- Zu, T., Gibbens, B., Doty, N. S., Gomes-Pereira, M., Huguet, A., Stone, M. D., Margolis, J., Peterson, M., Markowski, T. W., Ingram, M. A. C., Nan, Z., Forster, C., Low, W. C., Schoser, B., Somia, N. V., Clark, H. B., Schmechel, S., Bitterman, P. B., Gourdon, G., ... Ranum, L. P. W. (2011). Non-ATG-initiated translation directed by microsatellite expansions. *Proceedings of the National Academy of Sciences*, 108, 260–265. <https://doi.org/10.1073/pnas.1013343108>
- Zu, T., Liu, Y., Banez-Coronel, M., Reid, T., Pletnikova, O., Lewis, J., Miller, T. M., Harms, M. B., Falchook, A. E., Subramony, S. H., Ostrow, L. W., Rothstein, J. D., Troncoso, J. C., & Ranum, L. P. W. (2013). RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. *Proceedings of the National Academy of Sciences*, 110, E4968–E4977. <https://doi.org/10.1073/pnas.1315438110>

How to cite this article: Vangoor VR, Gomes-Duarte A, Pasterkamp RJ. Long non-coding RNAs in motor neuron development and disease. *J Neurochem* 2021;156:777–801. <https://doi.org/10.1111/jnc.15198>