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The long non-coding RNAs in neurodegenerative diseases: novel mechanisms of pathogenesis

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ABSTRACT

Background: Long-non-coding RNAs (lncRNAs), RNA molecules longer than 200 nucleotides, have been involved in several biological processes and in a growing number of diseases, controlling gene transcription, pre-mRNA processing, the transport of mature mRNAs to specific cellular compartments, the regulation of mRNA stability, protein translation and turnover. The fundamental role of lncRNAs in central nervous system (CNS) is becoming increasingly evident. LncRNAs are abundantly expressed in mammalian CNS in a specific spatio-temporal manner allowing a quick response to environmental/molecular changes. Methods: This article reviews the biology and mechanisms of action of lncRNAs underlying their potential role in CNS and in some neurodegenerative diseases. Results: an increasing number of studies report on lncRNAs involvement in different molecular mechanisms of gene expression modulation in CNS, from neural stem cell differentiation mainly by chromatin remodeling, to control of neuronal activities. More recently, lncRNAs have been implicated in neurodegenerative diseases, including Alzheimer's Disease, where the role of *BACE1-AS* lncRNA has been widely defined. *BACE1-AS* levels are up-regulated in AD brains where *BACE1-AS* acts by stabilizing *BACE1* mRNA thereby increasing BACE1 protein content and Aβ42 formation. In Frontotemporal dementia and Amyotrophic lateral sclerosis the lncRNAs *NEAT1_2* and *MALAT1* co-localize at nuclear paraspeckles with TDP-43 and FUS proteins and their binding to TDP-43 is markedly increased in affected brains. In Parkinson's Disease the lncRNA *UCHL1-AS1* acts by directly promoting translation of UCHL1 protein leading to perturbation of the ubiquitin-proteasome system. Different lncRNAs, such as *HTT-AS*, *BDNF-AS* and *HAR1*, were found to be dysregulated in their expression also in Huntington's Disease. In Fragile X syndrome (FXS) and Fragile X tremor/ataxia syndrome (FXTAS) patients, the presence of CGG repeats expansion alters the expression of the lncRNAs *FMR1-AS1* and *FMR6*. Interestingly, they are expressed in peripheral blood leukocytes, suggesting these lncRNAs may represent biomarkers for FXS/FXTAS early detection and therapy. Finally, the identification of the antisense RNAs *SCAANT1-AS* and *ATXN8OS* in spinocerebellar ataxia 7 and 8, respectively, suggests that very different mechanisms of action driven by lncRNAs may trigger neurodegeneration in these disorders.

The emerging role of lncRNAs in neurodegenerative diseases suggests that their dysregulation could trigger neuronal death via still unexplored RNA-based regulatory mechanisms which deserve further investigation. The evaluation of their diagnostic significance and therapeutic potential could also address the setting up of novel treatments in diseases where no cure is available to date.

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INTRODUCTION

Neurodegenerative disorders, in particular Alzheimer's disease (AD) and Parkinson's disease (PD), are increasing to epidemic proportions and, although the efforts carried out in the last decade by scientific community revealed important insights into their molecular bases, their etiology remains elusive. This condition reflects the high complexity of human brain biology. Since the great difference in cognitive abilities between humans and invertebrates is not correlated to a correspondent difference in the number of protein coding genes, it is more and more evident that additional mechanisms regulating alternative splicing and gene expression both at transcriptional and post-transcriptional levels play an important role in the development and function of the central nervous system (CNS) [1]. The fundamental role of non-coding RNAs (ncRNAs) in the fine regulation of gene expression in CNS, via control of nuclear processing of primary transcripts, transport of mature mRNAs to specific cellular compartments, as well as regulation of mRNA stability, translation and turnover, has become increasingly evident. The regulatory function of different classes of ncRNAs, such as microRNAs (miRNAs), piwi-interacting RNAs (piRNAs) and, more recently, long-non coding RNAs (lncRNAs), has been documented in a broad range of biological processes, including cell proliferation, differentiation, apoptosis, development and immune responses [2-4].

While the small ncRNAs have been widely characterized in the last decade as important players in CNS development and functioning and have also been associated to a variety of neurodegenerative diseases [5; 6], the specific functions of the lncRNAs, which have been primarily studied in the context of genomic imprinting, cancer, and cell differentiation, are only recently emerging as pivotal in CNS development and maintenance [7; 8].

A possible pathogenetic role of lncRNAs in neurodegenerative diseases has been suggested by the growing number of identified lncRNAs shown to regulate expression of genes involved in the neurodegeneration process or already associated to neurodegenerative disorders. We here report on the functions of lncRNAs and their implication in neurodegenerative diseases, considering their dysregulation as a yet poorly explored pathogenetic mechanism implicated in the neurodegenerative process. Furthermore, the evaluation of their diagnostic significance and therapeutic potential will be discussed, helping to address one of the major challenges in neurobiology, the setting-up of a treatment in diseases where no cure is available to date.

THE lncRNA BIOLOGY

LncRNAs are defined as RNA molecules longer than 200 nucleotides, generally lacking a protein-coding function [9] and, unlike miRNAs or piRNAs, they are quite heterogeneous, differing in size, interacting partners and mode of action. The estimated number of the human lncRNA genes, reported in the current release of NONCODE Ev4 ID (http://www.noncode.org/analysis.php), is 90.062, a number significantly

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higher than that of coding genes, now amounted to 19.815, according to the current GENCODE version 24 release (http://www.gencodegenes.org/stats/current.html). Localization of lncRNAs is mainly nuclear, but they can also shuttle in the cytoplasm. Although the signals driving cytoplasmic localization are at now unknown [10-12], an RNA motif consisting of the pentamer sequence AGCCC has been recently reported to mediate the nuclear localization of *BORG* lncRNA [13]. LncRNAs can be broadly classified into two large categories, based on their position in respect to protein-coding genes: 1) intergenic lncRNAs, including pseudogenes, long intergenic non-coding RNAs (lincRNAs), and very long intergenic non-coding RNAs (vlincRNAs/macroRNAs); 2) coding gene-overlapping lncRNAs, represented by intronic lncRNAs, natural antisense transcripts (NATs), expressed non-coding regions (ENORs), enhancer RNAs (eRNAs), and promoter-associated long RNAs (PALRs) [14].

The lncRNA gene structure is similar to that of protein-coding genes, but lncRNAs show a bias for having just one intron and a trend for less efficient co-transcriptional splicing [15; 16]. A proposed mechanism of lncRNA evolution is gene duplication followed by different events leading to non-coding genes that express transcripts endowed with regulatory functions [17]. On the basis of mass spectrometry data reported by Banfai and Derrien, a small fraction (8%) of the previously annotated lncRNAs was found to produce detectable peptides [15; 18]. lncRNAs seem to have low translational potential even when ribosomes attempt to decode them. Given that for a number of predicted human protein coding genes no protein experimental data have been provided, we can hypothesize that some human putative lncRNA genes might fall in the group of genes encoding the missing human proteins, estimated to be about 18% of the human proteome (neXtProt release 19/9/2014). The few previously annotated lncRNA genes expressing transcripts undergoing translation encode small peptides (<100 aminoacids), whose biological significance is mostly unknown [19; 20].

The expression of lncRNAs loci show similar features of protein-coding genes: transcription is mainly carried out by RNA polymerase II, splicing occurs using the same consensus signals of coding genes and half of the lncRNAs are 5' capped and 3' polyadenylated. It has been also shown that several lncRNAs may be spliced at their 5' and 3' ends generating circular RNAs. However the functional significance of circularization, possibly increasing their stability, has not been yet assessed [21]. Some lncRNAs are known to be transcribed also by RNA polymerase III, but these events cannot be detected using the current whole transcriptome sequencing methods which are based on the enrichment of poly(A)-purified transcripts [22]. This might be a reason why the number of lncRNAs is expected to be higher than that currently estimated. Nevertheless, lncRNAs are on average expressed at a lower level than mRNAs, although variations may occur in the different classes [11; 12] and their expression shows a cell-type specificity higher than that of protein coding genes [23]. LncRNA gene expression is believed to be regulated by mechanisms similar to those of protein-coding genes, including control of their synthesis by transcription factors and histone

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modifications, and RNA splicing, but this area of lncRNA biology still remains quite unexplored [24-26]. A recent study, integrating ChIP-seq, RNA-seq and based-BRIC-seq RNA half-life data, highlighted how regulation of RNA degradation plays an important role in determining transcript levels of many genes [27]. Similarly, the stability of lncRNAs might have in some cases an important role in their modulation with a consequent effect of lncRNAs post-transcriptional regulatory function [28]. LncRNAs have similar half-life pattern of mRNAs [29]: lncRNAs with short half-lives (< 4 h) have generally a regulatory function, whereas lncRNAs with long half-lives (≥ 4 h) are involved in housekeeping roles [30]. The stability of lncRNAs seems to be associated with their physiological roles, but this issue was not sufficiently investigated, even if recent studies provided findings addressing the relationship between lncRNA stability and their biological function in specific pathways [31; 32]. The molecular mechanisms regulating lncRNA stability are not well known; the specific RNA decay elements usually located in the 3'UTR of mRNAs, including AU-rich elements (AREs) and Puf family protein-binding sites, were not demonstrated to have a role in lncRNA stability, even if a possible function of predicted AREs should be further investigated [28].

Although lncRNAs selective pressure is between the highest one of coding genes and that of the ancestral repeat sequences, which is considered to be under neutral selection, the promoters of lncRNAs are under very high selective pressure, with levels comparable to the promoters of protein coding genes [15; 25; 33-35].

Unlike miRNAs, they are not highly conserved and at now their sequence does not allow to identify consensus sites and associated specific functions, even if high numbers of correlated positions among multiple lncRNA sequence alignments suggest that these regions are under selective pressure to maintain a functional RNA structure [15]. Consistently, mammalian and zebrafish lncRNAs show short stretches of conserved sequence, hence suggesting a functional relevance. Similarly, both location and structure of lncRNAs seem to be conserved, even in the absence of a high sequence conservation [23].

MECHANISMS OF ACTION OF lncRNAS

LncRNAs, being a large fraction of noncoding transcripts, might represent an important source of molecular regulators in the eukaryotic nucleus, contributing to modulate gene expression at different levels. The role of lncRNAs in the fine tuning of gene expression may quickly respond to changes of environmental conditions or to gene silencing as part of a developmental program [23].

In the last decade an increasing number of studies highlighted the involvement of lncRNAs at all levels of nuclear architecture consistent with DNA replication, transcriptional and post-transcriptional regulatory mechanisms and epigenetic functions. LncRNAs show also important cytoplasmic functions concerning positive and negative regulation of both mRNA and nascent protein stability, regulation of translation and miRNA activity [24].

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An example of lncRNA involved in DNA replication and maintenance of telomere stability is *TERRA*, a transcript containing telomeric UUAGGG repeats able to sequester the single strand DNA-binding protein hnRNPA1 and essential for DNA replication [36; 37] (Figure 1 A). The lncRNA control of nuclear architecture and transcription depends on different biological mechanisms. One of these is the long range DNA looping formation [38], promoting inter-or trans-chromosomal interactions to bring together enhancer/promoter pairs, or different promoters separated by large stretches on the same chromosome, or on different chromosomes [39] (Figure 1 B). Nuclear lncRNAs, interacting with chromatin and chromatin modification complexes or regulating gene expression, generally show low stability, allowing a dynamic gene expression control in response to environmental signals [28]. Furthermore, some of the nuclear sub-compartments are assembled on lncRNAs, such as the nuclear-enriched abundant transcript 1 (NEAT1). NEAT1 has been shown to scaffold nuclear paraspeckles, mammalian-specific ribonucleoprotein bodies regulating mRNA splicing and maturation, through RNA sequestering [40; 41] (Figure 1 C). lncRNAs belonging to NAT subclass, unlike proteins, can also act in cis or trans to guide epigenetic modifier complexes to targeted sites, by pairing with DNA or mRNA sequences (Figure 1 D). The cis function consists in the recruitment of protein complexes to their site of transcription, conversely the trans activity leads to the formation of protein complexes by binding and sequestering transcription factors away from their targeted chromosomal regions which are their site of transcription [42].

The lncRNAs transcription can generate a molecular structure that may lead the formation or remodeling of nuclear domains, through the recruitment or sequestration of proteins already present in the nuclear compartment, influencing their interactions [43]. These interchromatin granules are often characterized by specific functions such as pre-mRNA splicing and maturation. Unlike cellular organelles, these domains are not membrane delimited, but they are characterized by the specific interactors forming them in a stable fashion, maintaining the components associated (Figure 1 E). They often form close to the sites of RNA components involved in transcription, thus functioning as molecular anchors [44]. A well known lncRNA-mediated biological event concerning transcription regulation and based on nuclear architecture modification is X chromosome-inactivation (Figure 1 F) as well as the imprinting activity of lncRNAs and their role in the fine regulation of epigenetic modifications [24]. By means of lncRNA regulatory function, the transcriptional status of an entire chromosome can be controlled in a regional-, locus- or even allele-specific manner, through the recruitment of chromatin modifiers to the site of transcription. These complexes can create a local chromatin environment that may promote or inhibit the assembling of other regulators [45].

At cytoplasmic level the lncRNAs can control mRNA and protein stability, as well as mRNA transport and localization (Figures 1 G and H). The activity of *TINCR* (terminal differentiation-induced ncRNA) is an example of a lncRNA involved in post-transcriptional regulatory mechanims. *TINCR* is required during

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epidermal differentiation for the induction of specific key mediators. It localizes in the cytoplasm and, through the interaction with Staufen 1 protein (STAU1), promotes the stability of mRNAs containing the TINCR box motif [2]. LncRNAs can also have a role in the regulation of translation through the binding of the sense mRNA and consequent impairment of ribosome entry or arrest of translation between the initiation and elongation steps. In this context *lincRNA-p21* was reported to interact with the DDX6 (DEAD box helicase 6) translational repressor in particular conditions, repressing the translation of its mRNA targets [46] (Figures 1 H). On the contrary, an instance of positive regulation of translation is provided by the antisense *Uchl1* lncRNA (*UCHL1-AS1*), which has been shown to increase the UCHL1 protein level without leading to a change of the mRNA level [47]. *UCHL1-AS1* overlaps the first 73 nucleotides of *UCHL1* mRNA and contains two embedded repetitive sequences, one of which, SINEB2, is necessary for the induction of protein translation [47]. This repetitive sequence is present in many other lncRNAs from FANTOM3 database, suggesting a potential role of these RNA species in positively regulating translation of target transcripts [48].

LncRNAs were demonstrated to interact also with miRNAs and their target sites thereby modulating miRNA activity [49]. Interestingly, a number of lncRNAs has been reported to act as miRNA sponges, reducing the molecules available to bind their mRNA targets [24]. These RNA cross-interactions suggest the presence in the cell of a ncRNA network where these molecules mutually regulate themselves by sequestering each other temporarily, or by degrading each other through dsRNA formation [21; 50] (Figure 1 I).

The current knowledge on the lncRNAs mode of action shows how these RNA molecules play important roles at different levels of gene expression regulation, particularly when a fine tuning of a specific gene transcription is necessary to produce a fast response to stimuli of different origin. This regulatory function is consistent with mechanisms leading to a short lncRNA half-life and with a number of lncRNAs that currently seem to be higher than that of protein-coding genes (NONCODE Ev4 ID http://www.noncode.org/analysis.php), providing the nucleus with a very complex RNA-based system aimed at regulating coding-gene expression [15]. A regulatory mechanism based on an RNA-address code may act more rapidly and with a lower energy cost than a system based only on proteins [23]. In fact, lncRNAs do not need to be translated and/or transported into the cytoplasm and their targets are usually localized close to or overlapping with their own genomic loci. In contrast to the model of nuclear domains promoting molecular interactions, the spatial-based control can lead to the separation of the interactors until the appropriate moment. Environmental stresses may have as consequences the retention of specific proteins in the nucleolus, away from their normal site of action, thanks to the expression of specific noncoding RNAs [51]. According to the current knowledge on lncRNA functions, these RNA molecules are part of regulatory mechanisms for a fine control of gene expression able to respond to internal/external environmental changes, to manage complex biological events or developmental programs.

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LncRNAs IN NEURODEGENERATIVE DISEASES

Recent data highlight that lncRNAs are important in the development and functioning of the nervous system by both promoting the self-renewal of neural stem cells and neuronal differentiation, and by participating in the control of synaptic plasticity. Although a precise functional role in the nervous system has been attributed only to a few of them so far, lncRNAs have been described to be differentially expressed at specific stages during CNS development [52] or in specific cerebral regions [53].

It is therefore not surprising that misregulation of lncRNAs might play a pathogenetic role in neurodegenerative diseases. This view has been reinforced by the identification of a growing number of lncRNAs that directly regulate the expression of genes associated to neurodegenerative disorders, including Alzheimer's, Parkinson's, Huntington's diseases, Fragile X syndrome and Fragile X tremor/ataxia syndrome as well as spinocerebellar ataxia 7 or, by still unexplored mechanisms, the alternative splicing or mRNA transport/localization, as recently described in Frontotemporal dementia/Amyotrophic lateral sclerosis and spinocerebellar ataxia 8, respectively (Table 1).

In the following paragraphs, we will describe the diverse mechanisms reported for lncRNAs in neurodegenerative diseases and discuss how they could mechanistically be involved in the neurodegenerative processes.

Alzheimer Disease

Alzheimer disease (AD) is the most common neurodegenerative disease affecting aged population and, etiologically, it is a complex disorder with the majority of cases occurring as sporadic ones and only 10% showing family history. Genes associated to familial forms are mainly implicated in the processing and maturation of the amyloid precursor protein (APP) into the cleaved Aβ peptides which can form extracellular deposits in affected brains, the senile plaques, when the more amyloidogenic Aβ42 peptide is generated. This process is highly dependent on the correct cleavage of APP by the γ and β secretase enzymes. The antisense transcript of the β-secretase encoding gene *BACE1* (*BACE1-AS*) was reported to increase the stability of *BACE1* mRNA and to prevent binding of miRNA 485-5p, therefore positively regulating BACE1 protein content and promoting Aβ42 synthesis (Figure 2) [54]. Expression of *BACE1-AS* can be induced by different cell stressors, including treatment with Aβ42 itself, which suggests a feed-forward mechanism in AD pathogenesis [55]. Interestingly, *BACE1-AS* expression is elevated in AD patients brains together with the RNA-binding protein HuD which can bind both *BACE1* and *BACE1-AS* transcripts and increase their stability (Figure 2) [55; 56]. HuD can also bind and stabilize *APP* mRNA, thereby promoting Aβ42 peptide formation by increasing APP and BACE1 protein content as well as *BACE1-AS* lncRNA level [56]. This was observed both in cortical tissue from AD individuals and in transgenic mice over-

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expressing HuD protein. As a therapeutic approach, modulation of *BACE1* and *BACE1-AS* transcripts in APP transgenic mice by transient *in vivo* silencing was effective in reducing Aβ aggregation [57]. This potential therapeutic strategy targeting *BACE1* and *BACE1-AS* was also confirmed in a human neuroblastoma disease cell model where gene-silencing of *BACE1-AS* decreased the BACE1-dependent cleavage of APP with a consequent reduction of senile plaque formation [58].

A recent genome-wide search for differentially expressed lncRNAs in post-mortem human AD brains revealed a specific disease-associated signature with both up-regulated and down-regulated lncRNAs, mainly representing intergenic lncRNAs [59]. Although the mode of action of such deregulated lncRNAs is mainly unknown so far, it will be interesting to test whether such lncRNAs may serve as disease-specific biomarkers for AD. The expression of three specific lncRNAs, *17A*, *NDM29* and *51A* was reported to be upregulated in AD affected brains compared to healthy control brain tissues [60-62]. The lncnRNA *17A*, embedded in an antisense orientation in the third intron of the human G-protein-coupled receptor 51 (*GPR51,* also known as *GABBR2*) gene, was demonstrated to regulate *GPR51/GABBR2* pre-mRNA processing and favour the generation of the alternative and unfunctional splicing isoform B of the GABA B receptor (GABAB R2) (Figure 2) [60]. Indeed, the stable expression of *17A* in human neuroblastoma cells was shown to increase the synthesis of the GABAB R2 splicing isoform B that is defective of the intramembrane sequence peptide, thus generating non functional receptors unable to transduce GABAB-dependent intracellular signalling. In association to increased levels of the lncRNA *17A* and to a defective GABAB signaling, the secretion of the Aβ peptide was found to be induced with an increase in the Aβ42/Aβ40 ratio [60]. Similarly, the up-regulation of the lncRNA *NDM29* promoted Aβ secretion with an unbalance in the Aβ42/Aβ40 peptide ratio (Figure 2) [61]. Also the antisense ncRNA *51A*, deriving from the first intron of *SORL1* gene, a well recognized risk factor for AD, was described to promote the alternative splicing of *SORL1* and to increase Aβ formation [62]. Interestingly, both *17A* and *NDM29* ncRNA expression can be induced by inflammatory stimuli, which represent a pathogenic mechanism in AD, with the possibility of modulating such effect on Aβ synthesis by using anti-inflammatory drugs [60; 61].

The expression of the lncRNA *BC200/BCYRN1* (the human orthologue of *BC1*), which acts as a negative regulator of local protein synthesis at synapses, was reported to be altered in AD patients with opposite results, showing both down-regulation [63] and up-regulation [64] in affected brains. Despite this discrepancy in their observations, which probably reflects the different specific brain areas analyzed by the Authors, *BC200* is able to interact with different RNA-binding proteins known to control mRNA translation, including FMRP, PABP1 and SYNCRIP proteins, in post-synaptic dendritic microdomains (Figure 2) [65-67].

Frontotemporal dementia and amyotrophic lateral sclerosis

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Frontotemporal dementia (FTD) is the second important cause of dementia after AD in elderly population and, contrarily to the latter, it affects mainly the frontal and temporal lobes of the brain. Clinically, three different FTD subtypes can be distinguished: behavioral variant FTD, semantic dementia and progressive non-fluent aphasia, each accompanied by specific clinical features. FTD is a hereditary condition in nearly 40% of cases and several causative genes are associated to the disease, including *MAPT*, *PGRN*, *C9ORF72* and *VCP*. In the last few years increasing neuropathological and genetic findings have further supported the clinical observations of a disease continuum with motor neuron disease, in particular with amyotrophic lateral sclerosis (ALS). Neuropathological data, in fact, sustain the presence of abnormal cytoplasmic inclusions of the RNA-binding protein (RBP) TDP-43 in affected tissues of a subset of FTD patients (45%; FTD-TDP) and of nearly all ALS patients. Similarly, another nuclear RBP, FUS/TLS, forms cytoplasmic aggregates in affected tissues of 9% of FTD patients (FTD-FUS) and in familial ALS forms with mutations in *FUS/TLS* gene (~5%). Genetically, the pathological expansion of the hexanucleotide GGGGCC repeat in the first intron of *C9ORF72* gene represents the most frequent cause of both familial and sporadic FTD and ALS cases.

The lncRNAs *NEAT1_2* and *MALAT1* (*NEAT2*) were recently identified as RNA targets of TDP-43 by different binding assays both in experimental models and in human brains [68; 69]. *NEAT1_2* is essential for paraspeckle assembly [40], whereas *MALAT1* localizes at distinct nuclear sub-domains, the speckles, in association to different splicing factors [70]. Interestingly, binding of TDP-43 to these two lncRNAs was reported to be increased in human FTD brains compared to healthy controls [69]. Also FUS/TLS was shown to bind to *NEAT1_2* lncRNA although at a distinct site compared to TDP-43 and to associate to a consistent fraction (30%; 71/234) of all literature annotated lncRNAs [71]. Both TDP-43 and FUS co-localize with the lncRNA *NEAT1_2* in the nuclear paraspeckles in experimental cell lines, but *NEAT1_2* expression and localization in paraspeckels are increased in spinal motoneurons of sporadic ALS patients at early stages of disease [69]. Moreover, FUS loss-of-function was shown to impair paraspeckle assembly in disease cell models, while FUS-positive aggregates in the cytoplasm associated to FUS mutations induced sequestration of paraspeckle proteins [72].

In a different context, the RBP FUS/TLS was also described to interact with a series of lncRNAs transcribed from the 5' region of *CCND1* (*Cyclin D1*) gene in response to experimentally induced DNA damage [73]. Such lncRNAs-RBP binding determines a specific conformational change of FUS/TLS protein, making it able to bind to the histone acetyltransferases CBP and p300 and, as a consequence, to inhibit their activities as transcriptional co-activators [73].

Therefore in FTD and ALS diseases where the activity of TDP-43 and FUS is impaired affecting RNA metabolism at different levels, also the interaction of these two RBPs with lncRNAs may be defective, representing a novel pathogenetic mechanism to be explored.

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Parkinson disease

Parkinson disease (PD) is mainly a movement disorder, often accompanied by sensory and cognitive deficits or sleep problems, and is characterized by the specific neurodegeneration of the dopaminergic neurons in the substantia nigra. Similarly to AD and ALS, only a minor proportion of cases (5-10%) is familial with a wide genetic heterogeneity in both dominant and recessive disease forms. Several causative genes, including *Parkin*, *DJ-1* and *PINK1*, strongly support mitochondria dysfunction as one important pathogenetic mechanism in PD. PINK1 (PTEN-induced putative kinase 1) is involved in multiple aspects of mitochondria quality control and acts as a sensor of damaged mitochondria. By accumulating on the outer membrane of depolarized mitochondria, PINK1 is able to recruit also Parkin ubiquitin ligase on damaged mitochondria and to target them to degradation through autophagy. In line with a view of regulatory networks controlling target mRNA and protein content through epigenetic mechanisms, an antisense transcript of *PINK1* gene (*PINK1-AS*) was described to have a regulatory function on a short *PINK1* splicing isoform (*svPINK1*) encoding for the C-terminal region of the PINK1 protein with kinase activity [74]. The ncRNA *PINK1-AS* specifically and positively regulates the stability of the *svPINK1* transcript, although it is not clear yet the role of this short PINK1 protein isoform and its relevance in PD pathogenesis.

Also the antisense transcript of *UCHL1* (ubiquitin carboxy-terminal hydrolase L1), *UCHL1-AS1*, which is associated to PD both as a risk factor and as a rare causative gene, was recently identified in mice to exert its regulatory function on its target *Uchl1* mRNA at translational level [47]. UCHL1 protein is specifically expressed in the brain and in dopaminergic neurons where it is involved in the ubiquitin-proteasome system (UPS) by acting both in tagging proteins for degradation and in recycling ubiquitin molecules from degraded proteins. The lncRNA *AS-Uchl1* is mainly localized in the nucleus but, upon rapamycin treatment which inhibits mTOR pathway and CAP-dependent translation, it is induced to translocate into the cytoplasm. As a consequence, an increased association of its target *Uchl1* transcript to heavier polysomes and an increased translation of the UCHL1 protein are observed as strictly dependent on *AS-Uchl1* binding to its sense *Uchl1* mRNA [47]. It is interesting to note that the ability of the lncRNA *AS-Uchl1* to positively regulate translation of its sense transcript is due to the presence of the SINEB2 repetitive element [47; 48].

A recent RNA-seq approach in leukocytes and brain tissues from PD patients and controls revealed deregulation of specific lncRNAs, including the U1 spliceosomal lncRNA which was commonly up-regulated in PD blood and brain [75], suggesting its potential use as a disease biomarker.

Huntington's disease

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Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in exon 1 of huntingtin (*HTT*) gene, which generates a mutant huntingtin protein with an expanded polyglutamine tract at the N-terminus and potential toxic effects [76].

A natural antisense transcript, *HTT-AS*, was identified at the Huntington's disease repeat locus. The *HTT-AS* gene contains three exons and gives rise to two alternatively spliced isoforms: *HTT-AS_v1*, which is composed by exons 1 and 3 and includes the CAG repeat, and *HTT-AS_v2*, containing only exons 2 and 3. It was shown that the repeat expansion reduces *HTT-AS_v1* expression and, consistently, the levels of *HTT-AS_v1* are reduced in human HD frontal cortex. Moreover, *HTT-AS_v1* is able to negatively regulate *HTT* expression in a repeat length-dependent manner [77].

A role of *BDNF-AS*, a lncRNA transcribed starting from the brain derived neutrophic factor (*BDNF*) opposite strand, in the development of HD was also suggested. *BDNF* levels are known to be strongly downregulated in the brains of HD patients, possibly contributing to the clinical manifestations of the disease [78]. It was recently shown that *BDNF-AS* knock-down is able to induce *BDNF* upregulation [79]. In addition, *BDNF-AS* was demonstrated to inhibit *BDNF* transcription by recruiting the EZH2 catalitic subunit of the PRC2 silencing complex to the *BDNF* promoter, with consequent methylation of Lys27 of histone H3 (H3K27me2/3) [80; 81]. This evidence suggests a potential role of *BDNF-AS* dysregulation in HD pathogenesis.

Another lncRNA potentially involved in HD is *ABHD11-AS1*. The mouse ortholog, named *Abhd11os*, is enriched in the striatum and its expression levels are markedly reduced in different mouse models of HD. Interestingly, *Abhd11os* overexpression is neuroprotective against an N-terminal fragment of the mutant huntingtin, whereas *Abhd11os* knockdown is protoxic, indicating that the loss *Abhd11os* likely contributes to striatal vulnerability in HD [82].

Many other lncRNAs have been found to be dysregulated in HD and might play a significant role in HD neurodegeneration. Among them, the neural human accelerated region 1 (*HAR1*) lncRNA was shown to be repressed by REST (which is aberrantly localized to the nucleus in HD neurons) and to display significantly lower levels in the striatum of HD patients [83]. Another lncRNA found to be deregulated in HD is the *TUNA* (*Tcl1* upstream neuron associated) lincRNA, which is involved in pluripotency and neural differentiation of embryonic stem cells. The expression of *TUNA* in the striatum of HD patients was found to be associated with pathological disease severity, decreasing significantly as the disease grade increased [84]. Moreover, the analysis of microarray data revealed that *TUG1*, *LINC000341*, *RPS20P22*, *NEAT1* lncRNAs are upregulated in HD brains, while *MEG3*, *DGCR5*, *LINC000342* are downregulated [85]. It is worth noting that among these genes there are REST and p53 targets, and some of them are also known to interact with the PRC2 complex. Interestingly, the *NEAT1* lncRNA was also proposed to be involved in ALS pathogenesis (see above).

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Fragile X syndrome and Fragile X tremor/ataxia syndrome

Fragile X syndrome (FXS) is a genetic neurodevelopmental disorder characterized by moderate to severe intellectual deficit, macroorchidism and distinct physical features, which is caused by the expansion beyond 200 repeats of a CGG trinucleotide repeat in the 5'-untranslated region (5'-UTR) of the Fragile X mental retardation 1 (*FMR1*) gene [86]. This repeat expansion, called 'full mutation', causes *FMR1* upstream region to be abnormally methylated, resulting in *FMR1* transcriptional silencing and decreased protein levels in the brain. Fragile X tremor/ataxia syndrome (FXTAS) is a neurodegenerative disease whose pathogenesis is distinct from that of fragile X syndrome. FXTAS is caused by the expansion of the same CGG repeat in the *FMR1* gene, but, differently from FXS, the expanded repeat ranges from 55 to 200 units in size (the so-called 'premutation'), resulting in a toxic gain-of-function of *FMR1* RNA [87].

Different lncRNAs were recently discovered at the *FMR1* locus which display altered expression in FXS and FXTAS. *FMR1-AS1* (FMR1 antisense RNA 1), also known as *ASFMR1* or *FMR4*, is a primate-specific lncRNA overlapping the CGG repeat region, sharing the promoter with *FMR1* and transcribed in the opposite direction [88; 89]. The repeat expansion seems to affect transcription in both directions as, similarly to *FMR1*, the expression of *FMR1-AS1* was also found to be upregulated in FXTAS patients carrying the premutation and silenced in full mutated FXS patients. Interestingly, the *FMR1-AS1* premutated transcript exhibits a specific splicing isoform which is absent in the case of normal or full mutated alleles [88]. *FMR1-AS1* was not shown to have a regulatory activity on *FMR1* expression, but *FMR1-AS1* knockdown enhanced apoptosis while its overexpression increased cell proliferation, suggesting an anti-apoptotic function and a possible role in protection of neurons from death [89]. Furthermore, in differentiating human neural precursor cells (hNPCs), *FMR1-AS1* expression is developmentally regulated in opposition to expression of both *FMR1* and *MBD4* (methyl-CpG-binding domain protein 4), a *FMR1-AS1*-responsive gene, indicating a likely gene-regulatory function of this lncRNA during normal development [90]. Very recently, *FMR1-AS1* was also found to be a chromatin-associated transcript and it was shown to alter the chromatin state and the expression of several hundred genes *in trans*. Among these genes, there was an enrichment for those involved in neural development and cellular proliferation. Indeed, it was further demonstrated that *FMR1-AS1* may promote cellular proliferation of hNPCs [91]. It was also proposed that altered *FMR1-AS1* expression might contribute to some clinical aspects of FXS/FXTAS [89].

In addition, Pastori and colleagues [92] identified two further lncRNAs mapping to the *FMR1* locus: *FMR5* and *FMR6*. *FMR5* is a sense transcript localized upstream of the *FMR1* promoter and found to be expressed at low levels in both FXS/FXTAS patients and healthy individuals' brains, without significantly differences between full mutation, premutation and control samples. *FMR6* is an antisense transcript overlapping the 3' region of *FMR1*, which is specifically silenced in both FXS and FXTAS patients. Since these two lncRNAs

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were expressed in peripheral blood leukocytes, they were proposed as useful biomarkers for FXS/FXTAS early detection and therapeutic intervention [92].

Spinocerebellar ataxia

Spinocerebellar ataxias (SCAs) are a complex group of clinically and genetically heterogeneous neurodegenerative diseases characterized by progressive cerebellar ataxia in association with a broad spectrum of neurological and other clinical conditions.

Spinocerebellar ataxia type 8 (SCA8), a slowly progressive ataxia, is caused by an expanded CTG/CAG trinucleotide repeat (usually 80-250 units) on chromosome 13q21. Two genes transcribed in opposite directions encompass the repeat: ataxin 8 (*ATXN8*), encoding a polyglutamine protein in the CAG direction, and ataxin 8 opposite strand (*ATXN8OS*), whose transcription produces a noncoding RNA with a CUG expansion [93]. The *ATXN8OS* lncRNA containing the CUG repeat expansion was demonstrated to accumulate in ribonuclear inclusions along with the RBP MBNL1, and to cause splicing changes and increased expression of the GABA-A transporter 4 (GAT4/Gabt4), suggesting the involvement of toxic gain-of-function effects of the expanded-repeat *ATXN8OS* transcript in SCA8 pathogenesis [94]. Moreover, it was shown that the expression of human *ATXN8OS* in Drosophila induces late-onset neurodegeneration of the retina [95]. This effect was seen following the overexpression of both the wild-type and the repeat-expanded lncRNA, but it was proposed that the CUG expansion could alter the binding of *ATXN8OS* to RBPs. In particular, *ATXN8OS* was demonstrated to interact with Staufen, an RBP conserved in Drosophila and humans that mediates mRNA localization and transport in the nervous system. It is interesting to note that the *ATXN8OS* binding domain maps to the region which is expanded in SCA8 patients, suggesting that the interaction with Staufen could be compromised by the CUG repeat expansion, leading to disease [95].

Spinocerebellar ataxia type 7 (SCA7) is a neurodegenerative disorder due to a CAG trinucleotide/polyglutamine repeat expansion (usually 36 or more repeats) in the ataxin 7 (*ATXN7*) gene [96]. Larger CAG-repeat expansions are associated with an earlier disease onset and a more severe disease progress. A lncRNA, *SCAANT1* (SCA7/ATXN7 antisense RNA 1), was identified in the ataxin-7 promoter region which is transcribed antisense to the *ATXN7* gene starting from an alternative promoter. Loss of *SCAANT1* was shown to derepress ataxin-7 sense transcription from the alternative promoter and was accompanied by chromatin remodeling. On the contrary, upregulation in *cis* of *SCAANT1* transcription caused down-modulation of *ATXN7* and was accompanied by post-translational modification of histones [97]. These data demonstrated the involvement of the *SCAANT1* antisense lncRNA in the regulation of *ATXN7* expression, also indicating a likely contribution of lncRNA-mediated altered epigenetic regulation to SCA7 disease pathogenesis. Another lncRNA, *ATXN7L3B* (also known as *lnc-SCA7*), was shown to interact directly with *ATXN7* mRNA. The nature of this interaction is likely to be regulatory, since mutations in

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ATXN7 disrupt the interaction and result in a neuron-specific increase in *ATXN7* expression, predominantly in the SCA7 disease-relevant tissues, the retina and cerebellum, contributing to the selective neurodegeneration observed in SCA7 [98].

CONCLUSIONS AND FUTURE DIRECTIONS

The role of lncRNAs in the pathogenesis of neurodegenerative disorders is only beginning to be explored, but promises to open a new scenario for the setting up of innovative therapies for diseases for which no effective cure is available.

The great variety of lncRNA mechanisms of action and the relatively limited number of studies on lncRNAs in specific neurodegenerative diseases make it difficult to assemble a precise and conclusive picture of their involvement in these disorders. This notwithstanding, some recurrent pathomechanisms are clearly emerging from the literature data. Among them, the most straightforward is the dysregulation of lncRNAs which can in turn affect the expression of genes involved in the neurodegenerative process (e.g. *BACE1*), acting at different levels (chromatin structure, transcription, mRNA stability, translational efficiency, miRNA binding). This is usually the case, for instance, of many antisense transcripts transcribed from the same locus of the sense gene. Another group of lncRNAs deserving particular attention is composed by those lncRNAs which are able to interact with components of the epigenetic machinery, since their deregulation potentially alters the chromatin state and the expression of several genes, affecting multiple pathways involved in neurodegeneration. Another property of lncRNAs which can be relevant in triggering the neurodegenerative events is their ability to interact with RNA-binding proteins (RBPs) because, again, their dysregulation may impact the processing, stability, translation and localization of many different target transcripts. In some cases, the accumulation of a given lncRNA may have a toxic effect, as in the case of repeat expansion diseases. In other cases, our knowledge is limited to an evidence of lncRNA expression changes in the brains of affected patients compared to the healthy individuals and functional studies aimed at shedding light on their involvement in the neurodegenerative diseases are missing.

The elucidation of the lncRNA-mediated mechanisms of action is fundamental to better understand their role in brain functioning and their implication in the occurrence of neurodegenerative conditions. For this purpose, an effective strategy will be to study the effect of knocking-out or knocking-down specific lncRNAs in suitable model organisms and cell lines. Moreover, reports on the role of lncRNAs in cancers and malignant diseases of the CNS such as glioblastoma which are both numerous, and age-related and neurodegenerative in nature [8], may help shed light on the mechanisms of lncRNAs in neurodegenerative diseases. The latest transcriptome sequencing technologies, along with the development of suitable bioinformatic tools for identifying new potentially functional lncRNAs, will allow to expand the repertoire of known lncRNAs and to identify those which are dysregulated during the neurodegenerative processes. In

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addition, as our knowledge on the role of lncRNAs in the neurodegenerative disorders progresses, other evidence of dysregulated lncRNAs shared by different pathologies could also emerge, as in the case of *NEAT1*, uncovering common lncRNA-mediated pathomechanisms.

Importantly, the identification of lncRNA expression profiles robustly associated to the disease condition will prompt the use of lncRNAs as diagnostic and predictive biomarkers, especially if abnormal lncRNA expression patterns can be detected in blood and/or CSF of patients. In support of this perspective, the use of lncRNAs as diagnostic and prognostic markers is already successfully exploited in the field of oncology [8]. Moreover, a deeper understanding of the role of lncRNAs in neurodegenerative disorders will ultimately enable the development of innovative therapeutic strategies with lncRNAs as drug targets. A potential therapeutic intervention based on the reduction of the *UBE3A* antisense transcript (*UBE3A-ATS*) lncRNA using antisense oligonucleotides has been recently proposed for the treatment of patients affected by Angelman syndrome [99] and analogous oligonucleotide-mediated therapeutic strategies are also being developed by biotechnology companies for a wide range of disorders. Therefore we are confident that the experience gained in the above clinical fields will have also promising applications in the area of neurodegenerative diseases. In example, the targeted down-regulation of *BACE1-AS*, *17A*, *NDM29* and *51A* lncRNAs by specific antisense oligonucleotides could represent a potential therapeutic approach to reduce Aβ production in Alzheimer's disease as it already was shown to be effective in AD animal and cell models [57; 58; 60; 61]. Another conceivable strategy may consist in the pharmacological modulation of lncRNAs to modify consequently the expression of known target genes involved in neurodegeneration.

In conclusion, lncRNAs can be considered a great promise for one of the major challenges of the last decades, early diagnosis and cure of neurodegenerative diseases that are having an increasing social impact with the lengthening of human lifespan.

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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FIGURE LEGENDS

Figure 1: Regulatory mechanisms mediated by lncRNAs in the nucleus and in the cytoplasm

Nuclear functions of lncRNAs: A. The lncRNA *TERRA* maintains telomere stability during DNA replication; B. lncRNAs promote inter- or trans-chromosomal interactions regulating transcription of target loci; C. lncRNAs are able to scaffold nuclear paraspeckles, participating in the regulation of mRNA splicing and maturation; D. lncRNAs can also act in cis or trans to guide epigenetic modifier complexes to targeted sites, by pairing with DNA or mRNA sequences; E. lncRNAs can generate inter-chromatin granules involved in pre-mRNA splicing and maturation; F. lncRNAs have a role in X-chromosome inactivation by nuclear architecture modification. *Cytoplasmatic functions of lncRNAs*: G. lncRNAs can positively or negatively regulate target mRNA stability; H. lncRNAs can regulate protein levels by controlling their translation and stability; I. lncRNAs can interact with miRNAs acting as molecular sponge to modulate their activity .

Figure 2: Dysregulated lncRNAs and affected pathways in the pathogenesis of Alzheimer's disease. The *BACE1-AS* transcript increases the stability of *BACE1* mRNA and prevents the binding of miR-485-5p, positively regulating *BACE1* and promoting Aβ42 formation. *BACE1-AS* expression levels are elevated in AD and can be induced by Aβ42 itself, creating a feed-forward mechanism. Moreover, the RNA-binding protein HuD can bind both *BACE1* and *BACE1-AS* transcripts, increasing their stability. The lncnRNA *17A*, which is embedded in an antisense orientation within the *GABBR2* gene, is upregulated in AD and favours the synthesis of the GABBR2 receptor splicing isoform B that is defective in transducing the GABAB-dependent intracellular signalling. The up-regulation of *17A*, as well as of the lncRNA *NDM29*, promotes Aβ secretion with an increase in the Aβ42/Aβ40 peptide ratio. The antisense lncRNA *51A*, located in the first intron of *SORL1* gene, promotes the alternative splicing of *SORL1* and increases Aβ formation. Finally, the expression of the lncRNA *BC200*, which acts as a regulator of local protein synthesis at synapses, can be both down- or up-regulated in AD brains. *BC200* is able to interact with different RNA-binding proteins known to control mRNA translation in post-synaptic dendritic microdomains. The vertical arrows shown on the left of lncRNAs *BACE1-AS*, *NDM29*, *17A* and *BC200* indicate up- or down-regulation in AD.

Aβ= amyloid-beta peptide; APP= amyloid precursor protein; BACE1= beta-site APP-cleaving enzyme 1; BACE1-AS= BACE1 antisense RNA; BC200= brain cytoplasmic RNA of 200 nucleotides; GABBR2= gamma-aminobutyric acid (GABA) B receptor 2; HuD= Hu-antigen D/ELAV like protein 4; NDM29= neuroblastoma differentiation marker 29; RBPs= RNA-binding proteins; SORL1= sortilin-related receptor, L(DLR class) A repeats containing.

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Figure 1: Regulatory mechanisms mediated by lncRNAs in the nucleus and in the cytoplasm
Nuclear functions of lncRNAs: A. The lncRNA TERRA maintains telomere stability during DNA replication; B. lncRNAs promote inter- or trans-chromosomal interactions regulating transcription of target loci; C. lncRNAs are able to scaffold nuclear paraspeckles, participating in the regulation of mRNA splicing and maturation; D. lncRNAs can also act in cis or trans to guide epigenetic modifier complexes to targeted sites, by pairing with DNA or mRNA sequences; E. lncRNAs can generate inter-chromatin granules involved in pre-mRNA splicing and maturation; F. lncRNAs have a role in X-chromosome inactivation by nuclear architecture modification. Cytoplasmatic functions of lncRNAs: G. lncRNAs can positively or negatively regulate target mRNA stability; H. lncRNAs can regulate protein levels by controlling their translation and stability; I. lncRNAs can interact with miRNAs acting as molecular sponge to modulate their activity .

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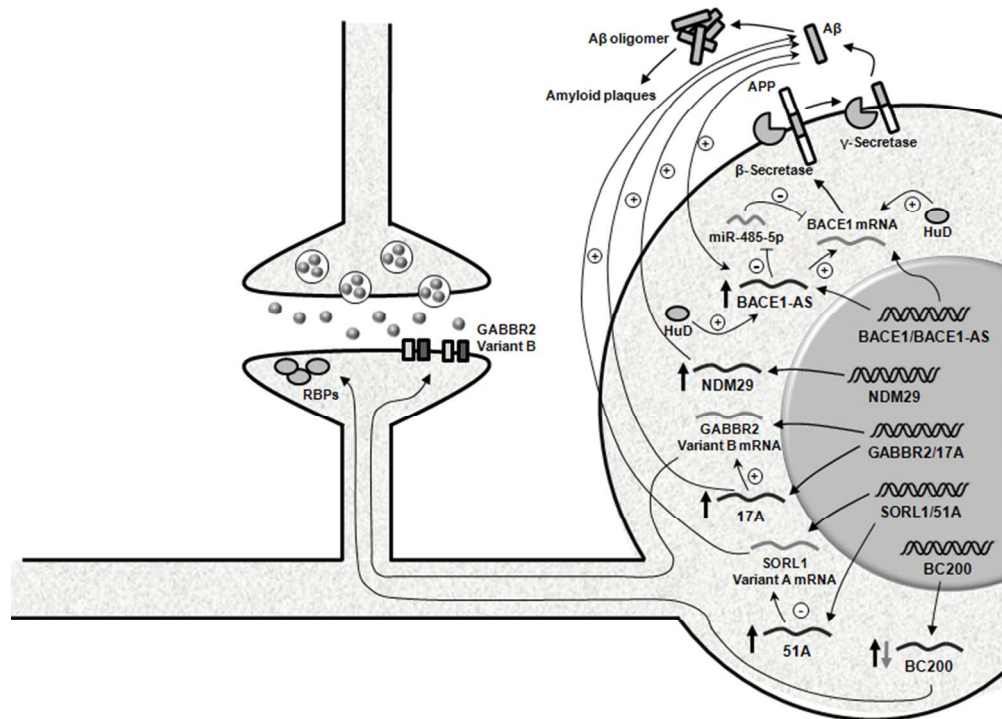51
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Figure 2: Dysregulated lncRNAs and affected pathways in the pathogenesis of Alzheimer's disease. The BACE1-AS transcript increases the stability of BACE1 mRNA and prevents the binding of miR-485-5p, positively regulating BACE1 and promoting Aβ42 formation. BACE1-AS expression levels are elevated in AD and can be induced by Aβ42 itself, creating a feed-forward mechanism. Moreover, the RNA-binding protein HuD can bind both BACE1 and BACE1-AS transcripts, increasing their stability. The lncnRNA 17A, which is embedded in an antisense orientation within the GABBR2 gene, is upregulated in AD and favours the synthesis of the GABBR2 receptor splicing isoform B that is defective in transducing the GABAB-dependent intracellular signalling. The up-regulation of 17A, as well as of the lncRNA NDM29, promotes Aβ secretion with an increase in the Aβ42/Aβ40 peptide ratio. The antisense lncRNA 51A, located in the first intron of SORL1 gene, promotes the alternative splicing of SORL1 and increases Aβ formation. Finally, the expression of the lncRNA BC200, which acts as a regulator of local protein synthesis at synapses, can be both down- or up-regulated in AD brains. BC200 is able to interact with different RNA-binding proteins known to control mRNA translation in post-synaptic dendritic microdomains. The vertical arrows shown on the left of lncRNAs BACE1-AS, NDM29, 17A and BC200 indicate up- or down-regulation in AD.

Aβ= amyloid-beta peptide; APP= amyloid precursor protein; BACE1= beta-site APP-cleaving enzyme 1; BACE1-AS= BACE1 antisense RNA; BC200= brain cytoplasmic RNA of 200 nucleotides; GABBR2= gamma-aminobutyric acid (GABA) B receptor 2; HuD= Hu-antigen D/ELAV like protein 4; NDM29= neuroblastoma differentiation marker 29; RBPs= RNA-binding proteins; SORL1= sortilin-related receptor, L(DLR class) A repeats containing.

Disease	lncRNA	Expression	Function	Ref
			induced by inflammatory processes	
	51A	Up	Promotes the alternative splicing of SORL1 and increases Aβ formation	[62]
	BC200/BCYRN1	Up/Down	Regulates translation by interacting with RNA-binding proteins	[63; 64; 65-67]
FTD/ALS	NEAT1	Up (early stages of ALS)	Role in paraspeckle assembly; binds to TDP-43 and FUS; increased binding to TDP-43 in FTD; expression and localization in paraspeckles increased in ALS motoneurons	[40; 68; 69; 71]
	MALAT1	-	Recruits splicing factors to paraspeckles; regulates phosphorylation of splicing factors	[68; 70]
Parkinsons's Disease	PINK1-AS	-	Positively regulates the stability of svPINK1 transcript	[74]
	UCHL1-AS1	-	Positively regulates UCHL1 mRNA translation	[47; 48]
	U1 spliceosomal lncRNA	Up	-	[75]
Huntington's Disease	HTT-AS	Down	Negatively regulates HTT expression	[77]
	BDNF-AS	-	Inhibits BDNF transcrption by recruitment of the PCR2 epigenetic silencing complex	[78-81]
	ABHD11-AS1	Down	Its overexpression is neuroprotective against an N-terminal fragment of the mutant huntingtin; its knockdown is protoxic	[82]
	HAR1	Down	-	[83]
	TUNA	Down	Involved in pluripotency and neural differentiation of embryonic stem cells; its expression in the striatum of HD patients decreases with the increasing of disease grade	[84]
	TUG	Up	Binds the PCR2 epigenetic silencing complex	[85]
	LINC000341	Up	-	[85]
	RPS20P22	Up	-	[85]
	NEAT1	Up	-	[85]
	MEG3	Down	Binds the PCR2 epigenetic silencing complex	[85]
	DGCR5	Down	-	[85]
	LINC000342	Down	-	[85]
FXS/FXTAS	FMR1-AS1	Down in FXS/Up in FXTAS	Anti-apoptotic function	[88-91]
	FMR5	-	-	[92]
	FMR6	Down in FXS/FXTAS	-	[92]
Spinocerebellar Ataxia	ATXN8OS	-	RNA toxic gain-of-function effects; altered binding to RNA-binding proteins	[93-95]