



CELLULAR AND MOLECULAR BIOLOGY

The implications of DNA methylation for amyotrophic lateral sclerosis

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Abstract: Amyotrophic lateral sclerosis (ALS) is a complex and serious neurodegenerative disorder that develops in consequence of the progressive loss of the upper and lower motor neurons. Cases of ALS are classified as sporadic (sALS), or familial (fALS). Over 90% of cases are sALS, while roughly 10% are related to inherited genetic mutations (fALS). Approximately 70% of the genetic mutations that contribute to fALS have been identified. On the other hand, the majority of the sALS cases have an undetermined genetic contributor and few mutations have been described, despite the advanced genetic analysis methods. Also, several factors contribute to the onset and progression of ALS. Numerous lines of evidence indicate that epigenetic changes are linked to aging, as well as neurodegenerative disorders, such as ALS. In most cases, they act as the heritable regulation of transcription by DNA methylation, histone modification and expression of noncoding RNAs. Mechanisms involving aberrant DNA methylation could be relevant to human ALS pathobiology and therapeutic targeting. Despite advances in research to find factors associated with ALS and more effective treatments, this disease remains complex and has low patient survival. Here, we provide a narrative review of the role of DNA methylation for this complex neurodegenerative disorder.

Key words: Amyotrophic lateral sclerosis, DNA methylation, neurodegenerative process, neuroepigenetics.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a complex and serious neurodegenerative disorder that develops in consequence of the progressive loss of the upper and lower motor neurons. ALS is the most common among the motor neuron diseases and is difficult to be diagnosed before the disease is clinically evident. Loss of lower and upper motor neurons results in muscle denervation, which leads to progressive muscle weakening. As a result, respiratory failure, difficulty in speaking and swallowing are observed, and eventually paralysis and death occur. Most patients show paralysis before bulbar symptoms, and the cause of death is usually related to respiratory failure typically

between one to five years from the time of disease onset, irrespective of the type/form of ALS (Rowland & Shneider 2001, Hardiman et al. 2011, Caballero-Hernandez et al. 2016). ALS incidence ranges from 0.6 to 3.8 per 100,000 person-years (Longinetti & Fang 2019), and the average age of ALS onset varies from 50 to 65 years old, with less than 5% of the cases showing an onset in individuals under 30 years old (Abhinav et al. 2007, Logroscino et al. 2010). ALS is a heterogeneous disease, with variable clinical presentation between patients, and is characterized by progressive motor deficits that evolve over weeks or months, (Talman et al. 2016). The disease is generally classified based on the site and pattern of the onset, and

the degree of involvement of upper and lower motor neurons (Theunissen et al. 2020). Cases of ALS are classified as sporadic (sALS), when they occur in people with no apparent history of the disorder in their family, or familial (fALS) when they occur in people with family history of ALS, or a related condition called frontotemporal dementia (FTD). Over 90% of cases are sALS, while approximately 10% are related to inherited genetic mutations (fALS) (Maruyama et al. 2010, Turner et al. 2013). Both fALS and sALS cases exhibit similar neuropathology. However, people with sALS usually first develop features of the condition in their late fifties or early sixties, while fALS symptoms and signs typically first appear in one's late forties or early fifties. Approximately 70% of the genetic mutations that contribute to fALS have been identified (Cook & Petrucelli 2019), but the majority of sALS cases have an undetermined genetic contributor and few mutations have been described, despite advanced genetic analysis methods (Nguyen et al. 2018). Several factors contribute to the onset and progression of ALS; in this article, we did a narrative review of the role of DNA methylation for this complex neurodegenerative disorder and Table I shows a summary of studies investigating DNA methylation related to ALS cited throughout this review.

DNA METHYLATION AND ALS

In 1993, SOD1 became the first gene linked to fALS (Rosen et al. 1993). After that, due to advances made in sequencing and molecular biological technologies, many other genes have been identified and currently, more than thirty genes and loci have been linked to fALS and sALS (Figure 1), as C9orf72, TARDBP, FUS, VCP, PFN1, TBK1, CHCHD10, TUBA4A, MATR3, CCNF, NEK1, C21orf2, ANXA11, and TIA1 (Nguyen et al. 2018). Genome-wide association studies have

shown more than 100 low-penetrance sALS loci, suggesting a polygenic inheritance model and a strong contribution of environmental factors in sALS (Van Rheenen et al. 2016, Ji et al. 2017).

The heritability of ALS is estimated to be around 50%, showing that a considerable portion of the risk could be conferred by environmental and lifestyle risk factors (Ryan et al. 2019). In addition to the identified genes and loci, there are epigenetic changes that may be associated with the development of ALS. Epigenetic changes in gene expression are mitotically or meiotically heritable and cannot be explained by changes in DNA sequence. In most cases, they act as the heritable regulation of DNA transcription by DNA methylation, histone modification and expression of noncoding RNAs. Epigenetic patterns can serve as biomarkers of disease progression and past exposures, such as smoking, alcohol intake, and exposure to environmental pollutants (Dupont et al. 2009).

DNA methylation (DNAm) is an extensively studied epigenetic modification that, in mammals, is predominantly found in cytosine-guanine dinucleotides (CpG). The formation of DNAm involves DNA methyltransferases (DNMTs), which add methyl groups onto carbon-5 of cytosine residues, hence forming 5-methylcytosines (5mCs). There are two classes of DNMTs: De novo DNMTs can newly methylate cytosines, setting up DNA methylation patterns (DNMT3a and DNMT3b), while maintenance DNMTs maintain these DNA methylation patterns (DNMT1). Chestnut et al. (2011) used a combination of cell culture and animal experimental designs to show that Dnmt activity can drive neuronal apoptosis. They showed that, in cultured spinal cord neurons, overexpression of Dnmt3a but not Dnmt1, induced degeneration; furthermore, robust upregulation of Dnmt1 and Dnmt3a was antecedent to neuronal apoptosis, induced by DNA damage. Authors suggest that disease

Table I. Summary of some studies investigating DNA methylation related to ALS cited throughout this review.

Reference	Sample Size*	Sample	Group	Methodology	Data
Morahan et al. 2009	20 (10+ 10)	Brain DNA	sALS	GWAS	Altered methylation of genes related to cell death pathways in sALS patients
Chestnut et al. 2011	24 (12 + 12) (Human tissue)	Mouse and Human CNS tissue	sALS	PCR	Motor neuron shows changes in DNMT's and 5mc in ALS patients
Figueroa-Romero et al. 2012	23 (12+ 11)	Human spinal cord tissue	sALS	ELISA; WGA	Identification of sALS epigenetic regulatory mechanisms
Wong et al. 2013	20 + 25 (mice); 4 (human CNS tissue)	Mice and Human CNS tissue	ALS	Pyrosequencing	mtDNA methylation patterns and DNMT3a levels are unnormal in skeletal muscle and spinal cord of presymptomatic ALS mice
Xi et al. 2013	177 (101+76)	Blood	C9ALS	Bisulfite sequencing	Higher degree of methylation is associated with a shorter disease duration in fALS
Tremolizzo et al. 2014	183 (96+ 87)	Blood	ALS	Incorporation of [(3)H]dCTP following HpaII cut	Increased DNA methylation as a marker in ALS dysfunction independently of age of onset
Liu et al. 2014	38 (32+6); 35 (cell lines)	Human tissue and peripheral DNA and cell lines	C9ALS and FTD	Bisulfite cloning and restriction enzyme-based methylation assays	Epigenetic silence of mutant C9ORF72 it might be protective
Russ et al. 2015	137 (118 + 19)	Brain samples and blood	ALS and FTD	qPCR	Hypermethylation is associated with prolonged disease survival
McMillan et al. 2015	45 (20+ 25)	Blood	C9ALS	Methylation-sensitive restriction enzyme DNA digestion coupled with quantitative PCR	C9ORF72 promoter hypermethylation have neuroprotective properties
Garton et al. 2017	229 (118+111)	Blood	ALS	Illumina HumanMethylation 450 array	Investigations on SOD1 and TARDBP did not suggest that DNA methylation signatures from blood are helpful in assessing functionality of these rare single-nucleotide variants.
Zhang et al. 2017	46 patients	Blood and CNS tissues	C9ALS and FTD	Infinium HumanMethylation 450k BeadChip	DNA methylation analysis of C9orf72 patients revealed that increased DNAm age-acceleration is associated with a more severe disease phenotype with a shorter disease duration and earlier age of onset.
Hamzeiy et al. 2018	550 (405+145)	Blood	sALS; fALS; C9ALS; SCA1 and 2; HD; FRDA; DM1	ELISA and direct bisulfite sequencing	Significant elevation in global 5-mC levels of about 2-7% on average for sALS and various forms of fALS
Stoccoro et al. 2018	144 (54 + 60)	Blood	ALS	MS-HRM; qPCR	Demethylation of d-loop it might be a compensatory mechanism for mtDNA upregulation in carriers of SOD1

Table I. Continuation.

Coppedè et al. 2018	35 (17+ 18)	Blood	fALS	ELISA ; MS-HRM	DNA methylation levels it might contribute to ALS phenotype of not fully penetrant SOD1 mutations
Tarr et al. 2019	4 (3+ 1)	Blood	fALS	EpiTYPER	High methylation age is a signature of ALS in older patients
Nabais et al. 2020	1.395 (782 + 613)	Blood	ALS	Illumina Infinium HumanMethylation450 Beadchip and MOMENT	Identification of 1 DMP between ALS cases and controls, which is annotated to CXXC5 on chromosome 5
Kim et al. 2020	49 (34+ 15)	Human post mortem CNS tissue	ALS	Pyrosequencing	Vulnerable neurons in human ALS accumulate DNA damage and activate and mobilize response effectors
Jackson et al. 2020	195 (108+87)	Blood	C9ALS	Quantitative methylation-sensitive restriction enzyme-based assays, digital molecular barcoding, quantitative real-time PCR, and Southern blotting	Elevated methylation levels, reduced expression levels and unstable expansions in C9ORF72 carries
Zhang et al. 2020	267 (249+ 18)	Blood and CNS tissues	ALS	GWAS	DNAm-age acceleration as biomarker
Appleby-Mallinder et al. 2021	124 (95+29)	Human post mortem spinal cord, motor cortex and anterior frontal cortex	sALS and C9ALS	Immunohistochemistry; Methylation EPIC array	DNA methylation contributes to LMN pathology
Nabais et al. 2021	9.894 (5.551 + 4.343)	Blood	ALS; PD; AD; SCZ1; SCZ2; RA	MOMENT	Shared differentially methylated positions in different neurodegenerative disorders
Zhang et al. 2021	249	Blood	ALS	Methylation EPIC BeadChip	16kb locus tagged by rs4970944 can be a modifier of ALS age of onset (each A-allele delays onset by 1.6 years)
Cai et al. 2022	64(32+32)	Blood	sALS	Infinium Methylation EPIC BeadChip	34 DMPs from 13 genes and 12 DMRs from 12 genes. Genes ELOVL2 and ARID1B was positively associated with the age of onset and disease duration, respectively.
Hop et al. 2022	10.462 (7.344+ 3.118)	Blood	ALS	EWAS	45 DMPs from 42 genes enriched for pathways related to metabolism, cholesterol biosynthesis and immunity pathways

*Sample size: Total (adversity group + control cohort);

Abbreviations: ALS: amyotrophic lateral sclerosis; CNS: central nervous system; sALS: sporadic amyotrophic lateral sclerosis; fALS: familiar amyotrophic lateral sclerosis; C9ALS: familial amyotrophic lateral sclerosis with G4C2 hexanucleotide repeat expansions in the first intron of C9ORF72; PD: Parkinson disease; AD: Alzheimer disease; FTD: Frontotemporal disorder; SCZ1 and 2: schizophrenia; RA: rheumatoid arthritis; HD: Huntington disease; FRDA: Friedreich disease; DM1: myotonic dystrophy type 1; GWAS: Genome wide association study; PCR: polymerase chain reaction; qPCR : quantitative polymerase chain reaction; EWAS: epigenome-wide association study; MS-HRM: Methylation-Sensitive High Resolution Melting; MOMENT: mixed-linear model method; DNMT's: DNA methyltransferases; mtDNA: mitochondrial DNA; DMPs: differentially methylated positions.

mechanisms involving aberrant DNA methylation could be relevant to human ALS pathobiology and therapeutic targeting (Chestnut et al. 2011).

Unusual DNAm patterns can be the consequence of environmental factors, as well as the cause or consequence of the disease. They have also been suggested to play a role in the beginning or evolution of neurodegenerative diseases, such as ALS (Nabais et al. 2020). DNAm is involved in proliferation and differentiation of neural stem cells, synaptic plasticity, neuronal reparation, learning, and memory (Fuso 2013).

In ALS patients, independently of the age of the disease onset, it was observed an increasing whole-blood DNA methylation, constituting a marker of epigenetic dysfunction in ALS (Tremolizzo et al. 2014). These findings regarding the elevation of global levels of 5-mC in sALS patients were replicated in another study (Hamzeiy et al. 2018). However, such studies differed from one another in respect to the magnitude of the increase. While Tremolizzo et al.

(2014) observed a considerably higher increase of about 25–30% in the global levels of 5-mC in blood for early- and late-onset sALS, Hamzeiy et al. (2018) reported an increase of about 2–3%. This high global DNA methylation is also seen in carriers of not fully penetrant SOD1 mutations (p.Asn65Ser, p.Gly72Ser, p.Gly93Asp, and p.Gly130_Glu133del), contributing to the ALS phenotype. In addition, a positive correlation between global DNA methylation levels and disease duration (months) was observed, adding to the evidence indicating a role for epigenetic factors modulating the phenotypic expression of the disease. (Coppedè et al. 2018).

In a previous study, methylation across the whole genome was examined in brain DNA of 10 sALS patients and 10 neurologically normal controls by Affymetrix GeneChip Human Tiling 2.0R Arrays. sALS patients had either hypo- or hyper-methylation at 38 methylation sites and of these, 23 were associated with genes and three with CpG islands. Pathway analysis showed that

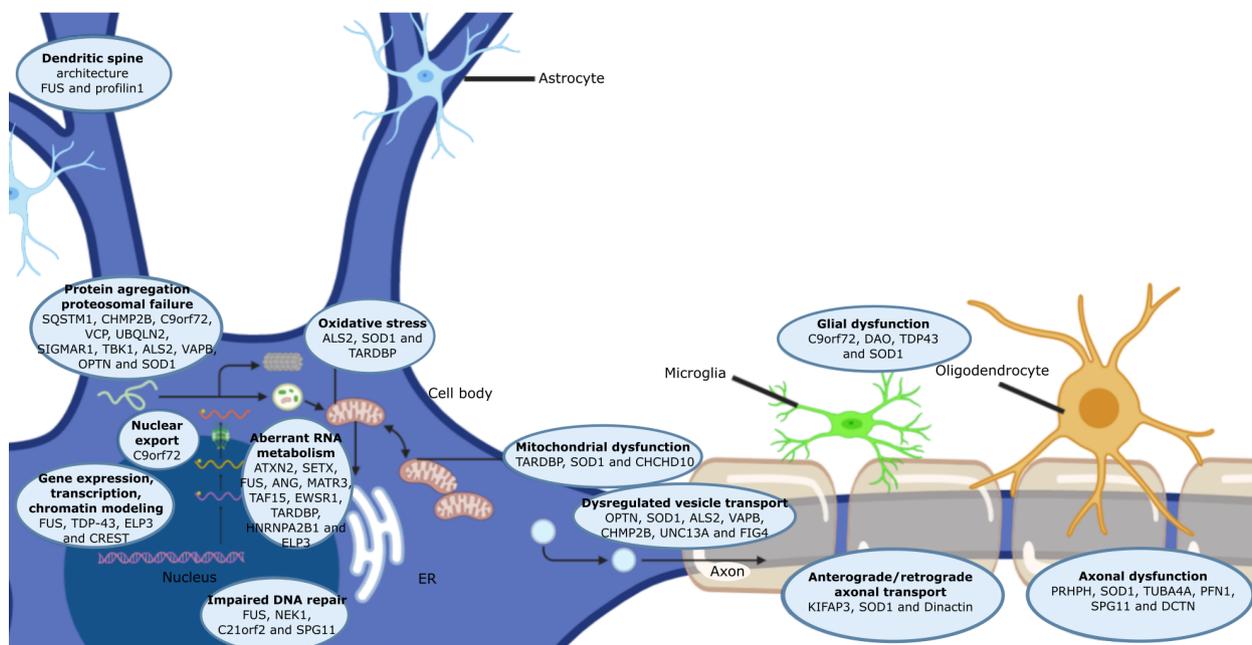


Figure 1. Genes related to ALS and impaired cell functions. In 1993, SOD1 was the first gene linked to fALS. Since then, multiple large, global studies have undertaken large sequencing and gene identification efforts, leading to the discovery of additional genes that are thought to cause or increase the risk of developing ALS. This figure summarizes some of these genes found in different studies since the discovery of SOD1.

genes with different methylation in sALS were particularly involved in calcium homeostasis, neurotransmission and oxidative stress (Morahan et al. 2009). Likewise, in a methylome-wide association study (MWAS) performed in postmortem spinal cord tissue of 12 ALS subjects and 11 age and gender-matched neurologically normal controls, authors reported 4261 significant differentially methylated positions (DMPs), annotated to 3574 genes. Functional enrichment analyses showed these genes to be involved in biological functions related to immune and inflammation response, suggesting that neurodegenerative processes in ALS may be associated with DNAm (Figuroa-Romero et al. 2012). In other studies, genome-wide analyses have found not only differential gene methylation in human ALS patients, but also alteration in Dnmt1, Dnmt3a, and 5-methylcytosine (Martin & Wong 2013, Garton et al. 2017). Moreover, for ALS patients, hypomethylation of the SOD1, VEGF, and GLT genes is typical (Bei 2017, Delgado-Morales & Esteller 2017).

Previous studies (Morahan et al. 2009, Figuroa-Romero et al. 2012) also revealed significant hypermethylation of genes involved in calcium dynamics, oxidative stress, and synapses, although most of the DNA methylation changes were found in non-promoter regions (intronic and cryptic). In a study by Kim et al. (2020), the authors used targeted gene promoter DNA methylation pyrosequencing (in CpG rich regions of promoter sequences) to examine the epigenetic status of base excision repair and DNA single-strand break repair. They identified the DNA repair genes *Ogg1*, *Apex1*, *Pnkp* and *Aptx* as hypomethylated in sporadic ALS, compared to age-matched control. This observation supports a role for changes in DNA damage accumulation and DNA damage response mediated by DNA methylation as possible pathological events in ALS (Kim et al. 2020).

Tarr et al. (2019) assessed genome-wide DNA methylation transcriptome (RNAseq) in a cohort of Australian monozygotic (MZ) twins (n = 3 pairs) and triplets (n = 1 set) that was discordant for ALS and represented sporadic ALS (2 pairs of MZ twins) and the two most common types of familial ALS, linked to C9orf72 (1 pair of MZ twins) and linked to SOD1 (1 pair of MZ triplets). They found statistically significant differential methylation in genes RAD9B and C8orf46 and similar results in an extended cohort of >1000 ALS cases and controls. The methylome analysis of monozygotic twins discordant for ALS, that identified the DNA damage response (DDR) gene RAD9B as differentially methylated, was consistent with the activation of DDR and DNA repair genes recently shown by Kim et al. (2020). Combined longitudinal methylation-transcription analysis within a single twin set implicated CCNF, DPP6, RAMP3, and CCS, which have been previously associated with ALS. Furthermore, Co-twin analyses indicated a significant interaction effect between age and disease status on DNA methylation age, with older twins showing a consistent difference between ALS-affected and unaffected co-twins in a longitudinal series. No differences were observed in C9orf72 methylation in the C9ALS MZ twin set (Tarr et al. 2019). Conversely, C9orf72 has been shown to have increased methylation and decreased transcription in ALS/FTD patients with the pathogenic repeat expansion, with methylation status considered fairly stable over time and within families. (Russ et al. 2015, Xi et al. 2013, Jackson et al. 2020, Hamzeiy et al. 2018).

Some studies for the C9orf72 gene showed that hypermethylation of the promoter region is a neuroprotective mechanism, preventing downstream molecular aberrations associated with the hexanucleotide repeat expansion (Liu et al. 2014, McMillan et al. 2015). About one-third of C9orf72 expansion carriers have a hypermethylated promoter region in the

cis-position relative to the mutation. Promoter hypermethylation of mutant C9orf72 was associated with transcriptional suppressing of C9orf72 in lymphoblast cell lines of ALS patients, resulting in diminished accumulation of intronic C9orf72 RNA and reduced numbers of RNA foci. The raise of mutant RNA is related with increased susceptibility to cellular stressors, together with oxidative and autophagic stress. On the other hand, C9orf72 demethylation results in oxidative and autophagic stress of mutant cells (Liu et al. 2014, McMillan et al. 2015).

Alterations in DNA methylation were also seen in post-mortem central nervous system tissues of ALS patients, in association with TDP43 proteinopathy. Investigators found that there were higher levels of methylation and hydroxymethylation in the residual lower motor neurons of both sALS and C9ALS (amyotrophic lateral sclerosis with G4C2 hexanucleotide repeat expansions in the first intron of C9ORF72) compared to the lower motor neurons of controls. Besides, in lower motor neurons from ALS cases, they found that neurons with pathological loss of TDP43 from the nucleus had lower levels of both 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC), in comparison to neurons with normal nuclear TDP43, suggesting a relationship between TDP43 proteinopathy and DNA methylation. They did not find a difference in the glial cell methylation of sALS and C9ALS and a small (if any) difference in the neurons of the motor cortex and middle frontal gyrus, suggesting the most significant changes in DNA methylation specifically affect lower motor neurons and no other cell types or neuron types (Appleby-Mallinder et al. 2021).

Recently, a blood-based epigenome-wide association study (EWAS) meta-analysis (Hop et al. 2022) in 10,462 samples (7,344 ALS patients and 3,118 controls) identified a total of 45 differentially methylated positions (DMPs) annotated to 42

genes, which are enriched for pathways and traits related to metabolism, cholesterol biosynthesis, and immunity. Interestingly, drivers of cholesterol biosynthesis enrichments included cg17901584 (DHCR24), cg06500161 (ABCG1), cg05119988 (MSMO1), and cg06690548 (SLC7A11). These genes are all involved in cholesterol biosynthesis and lipid transport. DNA methylation at these positions has been robustly linked to HDL (high density lipoprotein), total cholesterol triglyceride concentration, and body mass index-related traits such as diabetes and hepatic fat. Both the EWAS trait enrichments and PMS analyses indicate that lower body mass index is associated with ALS. Moreover, in the analysis of DMPs, the authors found that 5 of the 45 DMPs investigated correlated with survival in ALS and 4 of these were also related to disease progression (FKBP5, ATP8B2, SPIDR, and DHCR24). Furthermore, 8 out of the identified 45 sites were previously identified in another study (Nabais et al. 2021) on shared DNA methylation alterations across Parkinson's disease, Alzheimer's disease and ALS. Hop et al. (2022) speculated that these sites represent shared pathways involved in neurodegeneration and therefore, could have clinical utility. Another recent study (Cai et al. 2022) investigated the role of DNA methylation in sALS using whole blood of sALS patients. DNA methylation profiles were generated using Infinium MethylationEPIC BeadChip in 32 sALS patients and 32 healthy controls. They identified 34 significant differentially methylated positions (DMPs) in sALS patients' DNA, compared with the healthy controls related to 13 genes. Of these DMPs, five were hypermethylated and 29 were hypomethylated. They also identified 12 differentially methylated regions (DMRs), related to 12 genes (NWD1, LDHD, CIS, IQCE, TNF, PDE1C, LGALS1, CSNK1E, LRRC23, ENO2, ELOVL2, and ELOVL2-AS1). Two genes (DNAH9 and TNF) seem to be involved in the ALS pathway. They evaluated

the correlation between clinical features and DNA methylation profiling, finding that the methylation level of ELOVL2 and ARID1B were positively associated with the age of onset ($r = 0.86$, adjust $P = 0.001$) and disease duration ($r = 0.83$, adjust $P = 0.01$) respectively (Cai et al. 2022).

Epigenetic modifications of nuclear DNA have been studied in ALS and little is known concerning the epigenetic modifications in mtDNA. However, mitochondrial dysfunction is a feature of neurodegeneration, and some researchers have suggested that epigenetic modifications of the mtDNA, the so-called 'mitoepigenetics', might occur in ALS and other neurodegenerative conditions. Yet, the evidence for this hypothesis has remained scarce (Blanch et al. 2016, Stocco et al. 2017). Increased methylation of the mitochondrial gene coding for the 16S rRNA was detected in spinal cord neurons and skeletal muscle myofibrils of ALS transgenic mice (Wong et al. 2013). A recent work investigated mtDNA copy number and D-loop region methylation in carriers of SOD1, TARDBP, FUS and C9orf72 mutations, and observed increased mtDNA copy number in ALS patients, particularly in those with SOD1 or C9orf72 mutations. Also, SOD1 mutation carriers showed a significant decrease in D-loop methylation levels, which is expected since the D-loop region is critical for mtDNA replication and transcription (Stocco et al. 2018). The methylation levels of other mtDNA regions than the D-loop have been scarcely investigated in biological samples from patients with neurodegenerative disorders. Additional studies are required to clarify the biological significance of the mitoepigenetics and their cause/consequence relationship with the neurodegenerative process.

Age related diseases may occur because changes in DNA methylation and other epigenetic factors. These changes in DNA methylation can be a consequence of aging being seen in autophagy,

mitochondrial dysfunction, and problems in the homeostasis of proteases, which causes aggregation and mislocalization of proteins (Jiang & Guo 2020). All these issues are present in ALS but not always directed to DNA methylation aging, such as in ALS related to TDP43, VAPB and FUS, that show mislocalization and aggregation of proteins. The assessment of DNAm levels at age-related CpGs gave origin to DNAm clocks that may reflect biological aging and have been studied in ALS (Weidner et al. 2014, Jung & Pfeifer 2015, Zhang et al. 2017, Zhang et al. 2020).

In C9ALS, a significant correlation between aging and DNA methylation was found. The accelerated DNA methylation age was correlated with disease severity (Zhang et al. 2017). Correlation between CpG and levels of methylation were not found in this study. The paper also suggests that DNA methylation effect occurs by a group of CpG with minor effect, instead of one with a large effect. Authors emphasized that more studies around C9ORF72 and diseases variations related to these subjects are needed to authenticate this correlation (Zhang et al. 2017). Recently, in another study, authors showed that blood/ central nervous system-based DNAm-age acceleration was significantly associated with age of onset and survival in genetically unexplained ALS patients, suggesting a novel epigenetic modifier. In C9ALS patients, they observed a tendency for a stronger association of survival with DNAm-age acceleration vs a general ALS cohort (Zhang et al. 2020).

Since Single nucleotide polymorphisms (SNP's) were described as altering some CpG's sites, and these have an impact on DNA methylation and consequently gene expression (Bonder et al. 2017, Garton et al. 2017), some hypotheses were made around SNP's in genes related to ALS. Garton et al. (2017) investigated if altered methylation exists in other variants of ALS besides C9ORF72. They hypothesized

that carriers of ALS would have hypo or hyper methylation at the same genes relative to non-carriers. In this study, it was found two variants in SOD1 and two in TARDBP, although none of the genes showed strong evidence for SNP's acting in differential methylation in blood DNA. More studies are needed to investigate the reports of SOD1 and TARDBP as well as to explain altered methylation by SNP's (Garton et al. 2017). A recent CpG-SNP study showed that rs4970944 genotypes in C9ORF72 patients were correlated with delay in the onset of ALS (each A-allele delays onset by 1.6 years), due to the diminished expression of cerebellar expression of CTSS gene (encodes cathepsin S protein playing a key role in antigen presentation). Such findings highlight the role of immune processes and the genetic variants in the disease (Zhang et al. 2021).

CONCLUSIONS

DNA methylation is critical for the normal development and functioning of the human brain, such as the proliferation and differentiation of neural stem cells, synaptic plasticity, neuronal reparation, learning, and memory. Increasing reports about ALS indicate a high association between DNA methylation profiles and different clinical outcomes. In this review, we aim to discuss the latest evidence concerning DNA methylation alterations in ALS. Table I shows a summary of studies investigating DNA methylation related to ALS cited throughout this review. Together, these studies suggest that epigenetic regulation of biological functions via gene expression, such as DNA methylation, may be an important modifier in determining disease characteristics, since ALS has phenotypic heterogeneity.

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Adriana Helena de Oliveira Reis had the idea for the article, wrote the first draft, commented and critically revised on previous versions of the manuscript and approved the final manuscript. Luna Borges Figalo formatted the references according to the An Acad Bras Cienc, commented and critically revised on previous versions of the manuscript, and approved the final manuscript. Marco Orsini Figalo commented and critically revised on previous versions of the manuscript and approved the final manuscript. Bernardo Lemos commented and critically revised on previous versions of the manuscript and approved the final manuscript.

