Role of non-coding RNAs as biomarkers and the application of omics technologies in Alzheimer's disease (Review)

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Abstract. Alzheimer's disease (AD) is a neurodegenerative disorder that has a significant association with age. Despite its increasing incidence in the population, the etiology of the disease remains poorly understood, and there are currently no effective treatments readily available. The main genes that are associated with AD are the amyloid precursor protein, presenilin-1 and presenilin-2, as well as the apolipoprotein E gene. In addition to genetic factors, a wide range of environmental and lifestyle factors are equally characterized as risk factors for the development of AD, while non-coding RNAs (ncRNAs) and other epigenetic mechanisms play a key role in their detrimental effects. Multiple types of ncRNAs, such as microRNAs, circular RNAs, Piwi-interacting RNAs and long non-coding RNAs are being increasingly implicated in AD. Alterations in ncRNAs can be detected in cerebrospinal fluid, as well in as the brain, highlighting these as promising biomarkers for the detection and treatment of AD. Developments in high-throughput technologies have led to the so-called 'omics' era, which involves the collection of big data and information at both molecular and protein levels, while combining the development of novel computational and statistical tools capable of analyzing and filtering such data. The present review discusses the role of ncRNAs and their use as biomarkers for AD, and summarizes the findings from the application of omics technologies in AD.

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1. Introduction

Alzheimer's disease (AD) is a complex disorder characterized by the gradual loss of memory and the self-sufficiency of patients, the deterioration of thinking, and of the usage and understanding of written and spoken language, social isolation. It is also associated with behavioral changes, due to a confused state accompanied by apathy, depression and aggression (1-6). This disease occurs mainly in individuals >60 years of age; however, there is an increasing prevalence of the disorder in ~40% of the population <65 years of age (7). Considering this increasing prevalence of the disease, as well as the considerable socio-economic burden and the absence of any specialized treatment, it is important to make efforts to enhance the understanding of the pathophysiological mechanisms that lead to the development of AD (2,5,8).

AD occurs mainly sporadically without being due to a specific genetic background, with age being the main risk factor (1). The progressive atrophy of the cerebral regions of the hippocampus and cortex are representative macroscopic features of the disease and are clearly visible on a neuroimaging examination, while extracellular deposits of the amyloid-β peptide (Aβ1-42) and intraneuronal tangles of hyperphosphorylated forms of microtubule-associated protein

tau are some of the microscopic features of the disease. The activation of microglia associated with β-amyloid behavior, as well as the inflammatory response have been the focus of several studies on the contribution of β-amyloid cataracts to the development of AD (9-13).

Duing the early onset of AD, causal mutations in specific genes have been identified. The main genes are amyloid precursor protein (APP), presenilin-1 (PSEN1), and presenilin-2 (PSEN2). PSEN1 and PSEN2 are proteases involved in the conversion of APP into Aβ42, and are related neurotoxic products. The production of Aβ42 does not necessarily increase due to the abnormal variants of presenilin, but causes the production of other forms with a high tendency to cause agglomeration (14-16). On the other hand, the apolipoprotein E (ApoE) gene is the most well-known and important risk factor for the development of AD. This gene has three isoforms; however, 25% of AD cases carry the ε4 allele, while generally, the presence of two ApoE4 alleles increases the risk 10-fold compared to the presence of one allele (6,17). Although the mechanisms through which ApoE4 increases the risk of developing AD are not yet known, when this protein is poorly lipidated it binds to Aβ42 and is associated with its greater accumulation and oligomerization in the brain, as well as with reduced extracellular, microglial and the blood-brain barrier-mediated clearance of Aβ42. Thus, the destructive effect of Aβ42 on the function of synapses is aggravated (18-20).

A number of recent studies have focused on the search and detection of genetic loci or genes that are risk factors for AD, through the study and analysis of the genome (21). Genetic loci or genes, such as NME8, FERMT2, PICALM, PTK2B, CD2AP, CD33, CELF1SLC24A4/RIN3, FERMT2, CASS4 and DGS2 appear to be associated with the development of late-onset AD due to the single nucleotide polymorphisms (SNPs) that they contain (22). Aside from genetic factors, an array of environmental and lifestyle factors are known as risk factors for the development of AD, ranging from exposure to toxins to a high-cholesterol diet, while non-coding RNAs (ncRNAs) and other epigenetic mechanisms play a key role in their harmful effects (19,23,24).

Currently, various ncRNAs have been detected and studied in accordance with their involvement in AD. The main categories of these molecules are microRNAs (miRNAs/miRs), circular RNAs (circRNAs), Piwi-interacting RNAs (piRNAs) and long non-coding RNAs (lncRNAs). The functions of these molecules, and their ability to interact with each other, as well with DNA and proteins result in the regulation of gene expression, since they either promote or inhibit the expression of genes. In addition, their expression is altered due to a pathological condition, rendering them effective biomarkers for the early diagnosis of diseases, including cancer and neurodegenerative diseases.

The emergence of 'omics' technologies has revolutionized the study of complex pathologies and diseases, including AD. The applications of omics platforms involve the recognition and the study of genes (genomics), messenger RNAs (mRNAs, transcriptomics), epigenomic factors (epigenomics), proteins (proteomics), metabolites (metabolomics) and lipids (lipidomics). In addition, the interest of the gut microbiota (the microbiome/microbiomics) is increasing due to its association with various diseases (25). The analysis and combination of data derived from different omics technologies is crucial for complex pathologies, such as AD in order to acquire a complete knowledge of the disease. Cerebrospinal fluid (CSF) and blood were the main samples used in omics studies on patients with AD, with the former being in close contact with neurons, containing several soluble biomarkers of the brain, and thus reflecting the changes that occur during the disease (26,27). More specifically, the increase in total tau protein (t-tau) and phosphorylated tau protein (p-tau), as well as the decrease in Aβ42 in the CSF, reflect the formation of amyloid plaques and neurofibrillary tangles in the brain tissue, which are characteristics of AD. However, the etiology of AD depends on, and is due to multiple factors, including genetic alterations, proteins and ncRNAs (28).

Omics technologies are a promising tool for studying the multifaceted pathology of AD. With the advancement of technology, the era of omics enables the collection of diverse data, as well as the analysis and filtering of data, which is carried out through cutting-edge computational tools. The importance and value of omics and ncRNAs are highlighted through the process of the development of personalized diagnostic and therapeutic tools. For this purpose, various studies have focused on novel pathways and networks, demonstrating novel pathological mechanisms related to AD and linked to other diseases (29-31). The present review aimed to summarize the current evidence on the role and utilization of ncRNAs as biomarkers in AD, as well as to describe the application of omics technologies and large-scale data in the efficient prediction, diagnosis and treatment of AD.

2. Role of ncRNAs in AD

ncRNAs have several distinct classes. The most well-studied category is that of miRNAs, whose function is now well-understood. The role of miRNA epigenetic and genetic defects has been shown in a variety of diseases, including cardiovascular diseases (32), metabolic syndrome (33) and cancer (34) and pathologies of the nervous system, such as AD (35). However, in addition to miRNAs, there are several other classes of ncRNAs, such as small nucleolar RNAs (snoRNAs), circRNAs, piRNAs, Y RNAs and the large heterogeneous group of lncRNAs, key factors in the development of various human disorders, including AD, and with potential use as biomarkers (36) (Table I).

miRNAs. miRNAs are a class of small ncRNAs ~20-24 nucleotides in length, which bind to the 3'untranslated region (3'UTR) of target mRNAs, leading to post-transcriptional silencing either by transcription, degradation, or translational repression. To date, >2,000 miRNAs have been identified in the human genome that play a key role in critical biological processes (37), such as development, apoptosis, signal transduction and proliferation. With respect to the brain, they are expressed in neurons and are involved in processes of neuronal differentiation, synaptogenesis and plasticity (38). According to the literature, miRNAs have a significant impact on the development of several neurological diseases and disorders, such as AD, Parkinson's disease, amyotrophic lateral sclerosis and Huntington's disease (39,40).

Table I. ncRNAs in AD.

miRNAs	(Refs.)	circRNAs	(Refs.)	piRNAs	(Refs.)	lncRNAs	(Refs.)
miR-106a	(42)	ciRS-7	(71,81-83)	piR-38240	(92)	BACE1-AS	(100-103)
miR-520c	(42)	circHOMER1	(63,84)	piR-34393	(92)	NAT-Rad18	(104,105)
miR-20a	(43,44)	circCORO1C	(63,84)	piR-40666	(92)	51A	(105,106)
miR-19	(43,44)	circRNA KIAA1586	(63,85)	piR-51810	(92)	17A	(107)
miR-106b	(43,44)	circHDAC9	(63,86)	piR-hsa-1282	(92)	BCYRN1	(105,109)
miR-20a family	(43,44)	circRNA_0000950	(63,87)	piR-hsa-23538	(92)	AD-linc2	(111)
miR-101	(45)	circNF1-419	(63,88,89)	piR-hsa-23566	(92)	HAO2-AS	(111)
miR-147	(46,47)			piR-hsa-27400	(92)	EBF3-AS	(111)
miR-124	(48-50)			piR-hsa-27725	(92)	AD-linc1	(111)
miR-339-5p	(36)			piR-hsa-28116	(92)	MAGI2-AS3	(108)
miR-195	(61)			piR-hsa-28189	(92)		
miR-107	(62)			piR-hsa-28390	(92)		
miR-9	(64)			piR-hsa-29114	(92)		
miR-144/miR-451	(66)			piR-hsa-7193	(92)		
miR-181c	(71,72)						
miR-146a	(73-75)						
miR-298/328	(57)						
miR-135a	(58)						
miR-135b	(59)						
miR-455-3p	(76)						
miR-485-3p	(77)						

In the table, columns 1, 3, 5 and 7 contain the miRNAs, circRNAs, piRNAs and lncRNAs that are involved in AD, respectively. In a similar manner, column 2 contains the studies that are referring to each miRNA, respectively, column 4 contains the studies that are referring to each circRNA respectively, column 6 contains the studies that are referring to each piRNA, respectively, and column 8 contains the studies that are referring to each lncRNA, respectively. miRNAs/miRs, microRNAs; circRNAs, circular RNAs; piRNAs, Piwi-interacting RNAs; lncRNAs, long non-coding RNAs; AD, Alzheimer's disease.

As regards AD, miRNAs have been shown to be involved in Aβ pathology by regulating APP expression and other enzymes involved in Aβ processing, such as β-secretase (BACE1). The first study to record the regulatory role of miRNAs in APP mRNA involved the homologous APP gene in *C. elegans*, APL-1, which showed its developmental regulation by miRNA let-7 (41). Subsequent studies have demonstrated that APP is regulated by miRNA in humans, where the overexpression of miR-106a and miR-520c has been shown to lead to the translational suppression of APP mRNA, thereby significantly reducing APP levels (42). In addition, miR-20a, miR-19, miR-106b, the miR-20a family (43,44) and miR-101 (45) have been shown to directly regulate APP mRNA in human cells *in vitro*. In addition, the effect of SNPs on miRNA binding sites in the 3'-UTR of APP mRNA in AD pathology and the risk of AD are demonstrated, where more specifically, miR-147 and miR-20a were shown to be the affected SNPs variants associated with AD in the 3'-UTR of APP mRNA (46,47). Finally, miRNAs, such as miR-124, which regulates the expression of the polypyrimidine tract-binding protein 1 (PTB1) in neuronal cell lines, have also been implicated in the regulation of the alternative splicing of APP (48-50). In general, there is significant evidence of the increased levels of exon 7 and 8 isoforms of APP in brains of patients with AD, while abnormal APP splicing has been shown to be associated with an increased Aβ production (51,52).

The importance of BACE1 activity in AD lies in the fact that this factor cleaves APP as the first and rate-limiting step in the formation of Aβ (53). In this case, miRNAs belonging to the miR-29 family have been well-studied *in vitro* and *in vivo*. The three major mature miRNAs in this family are miR-29a, miR-29b and miR-29c, the latter of which has been shown to regulate BACE1 expression by targeting the 3'-UTR in both human and mouse cell lines (54-56). The aforementioned miR-29, as well as other miRNAs that directly target BACE1 *in vitro*, such as miR-298/328 (57), miR-135a, miR-135b (59), miR-9 (60), miR-298, miR-339-5p, miR-195 (61) and miR-107 (62), are deregulated in brains affected by AD, mainly exhibited a reduced expression (36,63).

miR-9 is another miRNA involved in Aβ regulation, targeting calcium/calmodulin-dependent protein kinase kinase 2 (CAMKK2), thereby attenuating Aβ-induced synaptic toxicity (64). In addition, this miRNA appears to be involved in insulin signaling, which may be associated with an increased risk of developing diabetes in patients with AD (65). The miR-144/miR-451, finally, has been shown to regulate α-secretase ADAM10, which protects the brain from the production of Aβ (66).

The search and discovery of specialized and effective biomarkers for the prediction and early detection of AD is of utmost importance for the better management of symptoms

and timely intervention (67,68). For this purpose, miRNAs have been proposed as promising candidate biomarkers due to their high stability under storage and handling conditions (69). Through qPCR and RNA-seq studies, it has now become possible to identify circulating miRNAs in plasma and CSF that serve as biomarkers for AD and to construct miRNA catalogs that are differentially expressed between AD and control groups to identify new biomarkers (70). Two miRNAs that have been identified as suitable biomarkers for AD are miR-181c (71,72) and miR-146a (73-75). The former was found to be downregulated in the serum and CSF of patients with AD, while the latter was found to be upregulated in brains affected by AD, as well as in the CSF of patients with AD. miR-455-3p is another potential biomarker for AD, as its level is higher in the serum of patients with AD (76), as well as miR-485-3p, whose expression was found to be upregulated in patients with AD and cell models (77).

circRNAs. circRNAs are a class of non-coding RNAs that originate primarily from the exonic regions of the genes encoding proteins. Their length is variable, while they display significant stability. These ncRNAs act as regulators of miRNAs, to which they bind through specific binding sites. circRNAs are expressed in central nervous system (CNS) tissue and tend to accumulate during the normal aging process of the brain, exhibiting susceptibility to age-related neurodegenerative diseases, such as AD. This renders them potential therapeutic targets and biomarkers for the diagnosis and treatment of AD (78-80).

A well-studied circRNA that has been linked to AD is ciRS-7. This RNA binds to the well-preserved miRNA-7, which is abundant in the human brain. More specifically, ciRS-7 contains several binding sites specific for miRNA-7 and acts as a 'sponge', thus inhibiting the functions of miRNA-7 (81). In the hippocampus of patients with AD, there is a downregulation of ciRS-7 and, consequently, of its activity as a miRNA-7 sponge, causing the latter to exhibit increased endogenous levels in AD (71,82). The upregulation of miRNA-7 has the ability to target and downregulate ubiquitin protein ligase, UBE2A, which is involved in the autophagic clearance of amyloid peptides in the brain affected by AD (83).

In addition, additional studies have reported two other circRNAs that are dysregulated in cortical areas in AD, namely circHOMER1 and circCORO1C. These ncRNAs are significantly associated with the neuropathological status of AD, as they bind two miRNAs, miR-651 and miR-105, respectively, which target both APP and SNCA42 and are associated with the pathology of AD (63,84). circRNA KIAA1586 is another circRNA that functions as a miRNA sponge, which specifically binds several miRNAs, including miR-29b, miR-101 and miR-15a, that regulate different AD-associated genes (63,85). Moreover, circHDAC9 binds miR-138 and its expression is decreased in AD, resulting in the downregulation of ADAM10 by miR-138, thus promoting Aβ production (63,86).

In this context, circRNA_0000950 functions as a miR-103 sponge, leading to the upregulation of the prostaglandin-endoperoxide synthase 2 and interleukin (IL)-6 and IL-1β, as well as tumor necrosis factor (TNF), and results in an increase in neuronal cell apoptosis (63,87). CircNF1-419 is related to early neuropathological changes and interacts with dynamin-1 and adaptor protein 2 B1. Its overexpression reduces the levels of AD marker proteins, such as tau, p-tau, Aβ1-42 and ApoE, and inflammatory factors, including TNF and the nuclear factor kappa B subunit 1, resulting in delayed senile dementia and AD progression (63,88,89). It is therefore clear that circRNAs may play a critical role in AD, mainly as miRNA sponges (63), where the inhibition of 'sponging miRNA activity', which translates to the upregulation of specific miRNAs, is a possible reason for the downregulation of important genes associated with the brain in AD (83).

piRNAs. piRNAs are also a class of ncRNAs that are ~24-34 nucleotides in length and are associated with AD, as they are involved in CNS stress and physical damage response. These ncRNAs interact with a specific family of Argonaute-associated 'MILI/MIWI' RNA-binding proteins and can affect the cytoplasmic translation of mRNAs into proteins, as well as the transcription of genes by influencing histones and the methylation of DNA (17).

In general, piRNAs appear to be overexpressed in neurodegenerative diseases (90). In the study by Qiu *et al* (91), 9,453 piRNAs were detected in the brains of patients with AD, and 103 piRNAs were associated with the risk of developing AD, of which 81 were upregulated and 22 were downregulated. piRNAs are considered a potential biomarker for AD due to their association with SNPs of genome-wide significant risk, such as ApoE (91). In addition, in the study by Roy *et al* (92), 146 piRNAs were found to be upregulated in patients with AD, while they were associated with five critical AD-related pathway targets. More specifically, the enrichment of the CYCS, LIN7C, KPNA6 and RAB11A genes was observed, regulated by four piRNAs, piR-38240, piR-34393, piR-40666 and piR-51810 (92). Finally, the analysis of two different AD datasets led to the identification of 10 overlapping, differentially expressed piRNAs, with potential as biomarkers for AD. These piRNAs include piR-hsa-1282, piR-hsa-23538, piR-hsa-23566, piR-hsa-27400, piR-hsa-27725, piR-hsa-28116, piR-hsa-28189, piR-hsa-28390, piR-hsa-29114 and piR-hsa-7193 (92).

lncRNAs. lncRNAs are another class of ncRNAs that has attracted scientific interest in the battle against neurodegenerative diseases. They are >200 nucleotides in length and can be derived from different regions of the genome, such as promoters, enhancers, introns, UTRs, overlapping or non-coding isoforms of coding genes, antisense (AS) to other transcripts and pseudogenes (93,94). Through technological advances and new laboratory techniques, a vast amount of information has been collected on the role of lncRNAs in a variety of vital biological processes (95), including transcription (96), alternative splicing (97), translation, apoptosis (98) and the cell cycle (99).

RNA sequencing methods have enabled the study of lncRNAs and their role in various diseases, including AD. In general, the majority of examples of lncRNAs whose activity has been studied belong to the subcategory of AS lncRNAs. A well-studied lncRNA with an elucidated involvement in AD is lncRNA BACE1-AS (100), which is transcribed from the complementary strand of the BACE1 gene. This lncRNA is in direct involvement with elevated levels of Aβ 1-42 in AD, as it drives the feed-forward regulation of β-secretase (63,101,102),

and it can bind to miR-214-3p, promoting autophagy-mediated neuronal damage through the miR-214-3p/ATG5 signaling axis in AD (103). In addition to BACE1-AS lncRNA, NAT-Rad18 (104,105) and 51A (63,105,106) are two other lncRNAs involved in AD, as the former has been shown to be upregulated in rat neurons in response to the Aβ peptide, and the latter affects the formation of Aβ and is upregulated in AD by overlapping it with SORL1. 17A is another AS lncRNA, which is complementary to an endogenous region of the GABA receptor gene. The expression of this lncRNA leads to the production of alternative splicing transcripts of this receptor which, in combination with its upregulation in AD, leads to increased Aβ secretion in neuroblastoma cells (107). According to the study by Zhang and Wang (108), the expression of MAGI2-AS3 lncRNA was increased in AD cell models. This lncRNA is a regulator of cell viability in diverse diseases. Its overexpression enhances the effects of Aβ25-35 on neuronal viability and neuroinflammation, and its knockdown reduces neurotoxicity and neuroinflammation, highlighting the potential role of MAGI2-AS3 in AD progression and treatment. Moreover, this lncRNA functions as an miR-374b-5p sponge, a miRNA that targets BACE1 mRNA and interacts with AKT1, RECK, WNT16, VEGFA, TACC1 and SRSF7 mRNAs, and with compounds including cisplatin, gemcitabine, tamoxifen and 5-fluorouracil (108). Long intergenic ncRNAs (lincRNAs) are another subcategory of lncRNAs that are abundant in the human genome. The primate-specific BC200 RNA (BCYRN1) is a lincRNA that may be involved in AD. This RNA was detected in the dendritic domains of neurons and its downregulation was observed during aging (63,105,109).

The critical role of lncRNAs in AD was first evidenced through the study of Zhou and Xu (110), which used an algorithm to analyze microarray data from the brain and identified ~100 lncRNAs that were altered in AD. Notably, a number of these lncRNAs were specific to the brain and could be used as biomarkers for AD, since altered expression signatures of lncRNAs provided the ability to predict AD with the same accuracy as the altered protein-coding genes, while requiring fewer lncRNAs for optimal prediction in comparison to protein-coding genes (110). In addition, in a study by Magistri *et al* (111), significant alterations in the lncRNA expression profile were observed in brains affected by AD, with the majority of the altered lncRNAs found to be intergenic. According to the results of that study, the AS lncRNAs, AD-linc2, HAO2-AS and EBF3-AS were dependent on neuronal activity, while AD-linc1 was upregulated in response to Aβ (111). These efforts are only the beginning of a long road toward the complete elucidation of the involvement of lncRNAs in AD, with further studies required to explore their potential as novel biomarkers and pharmacological targets.

In summary, the main ncRNAs that are involved in AD are classified into four categories, miRNAs, piRNAs, circRNAs and lncRNAs. These non-coding molecules function via several mechanisms, such as inhibiting or promoting the expression of genes that are associated with AD, or they can be used as effective biomarkers as their expression is altered in AD-affected brains. These two types of classification of those ncRNAs, based on their category and based on their function, are illustrated in Fig. 1.

3. Application of omics technologies in AD

The wave of 'omics' has been taking the scientific world by storm, encompassing genomics, transcriptomics, epigenomics, proteomics, metabolomics and lipidomics as part of a rounded approach (112). When faced with complex diseases, such as AD, the efficient analysis and integration of data that omics technologies yield is critical for the development of diagnostics and therapeutics (28,113,114).

Genomics. Genetics studies, genome-wide association studies (GWAS) and next-generation sequencing (NGS) technologies have helped to gain knowledge about the genes which are associated with a risk of developing AD. Genetic studies have enabled the detection of rare mutations in the genes of APP, PSEN1 and PSEN2, which are associated with the dominantly inherited early-onset AD, as well as the identification of genetic components that affect the development of the sporadic cases of the disease. In addition, genomics analysis has shown that gene-gene interaction can be a significant risk factor for the development of AD (115,116).

GWAS can lead to the identification of genes with common variants involved in various diseases. By comparing the whole genome set of genetic variants in different individuals, GWAS shed light on SNP characteristics of different diseases, highlighting the possibility of associations between the detected variants. Several genomic loci of interest have been identified that may increase the risk of an individual for developing late-onset AD, including genes involved in β-amyloid processing and clearance, immune response and inflammation, such as CR1, CD33, MS4A, ABCA7 EPHA1 and MEF2C, in the metabolism of cholesterol, such as ApoE, SORL1, ABCA7 and CLU, and in the regulation of endocytosis, such as BIN1, CD2AP, PICALM, EPHA1, SORL1 (117,118).

In addition to GWAS, NGS technologies, including whole genome sequencing and whole exome sequencing, enable the detection of rare mutations that affect complex diseases. Significant are the findings of NGS studies, which have identified new risk genes with low-frequency variants, including TREM2, which encodes the ADAM10 activation receptor expressed in myeloid cells and leads to defective α-secretory activity, phospholipase D3, UNC5C and AKAP9 (119,120).

Epigenomics. Non-hereditary epigenetic changes have the potential to equally affect the risk of developing AD. Epigenetic modifications mainly involve DNA methylation and histone modification, thereby regulating gene expression. In the case of AD, the reduced methylation of DNA is described, while at the same time, a number of genes that have been characterized in AD exhibit a high level of methylation in their promoters and in the cytosines that precede guanines (CpG islands) (121). Two large-scale epigenome-wide association studies have identified four new genetic loci, including RHBDF2, RPL13, C10orf54-CDH23 and ANK1, with differential methylation, suggesting an association with the risk of developing AD (122,123). However, histone modification studies have yielded conflicting results regarding histone acetylation levels in AD (124-127). Thus, the heterogeneity of results from epigenomics studies indicates that the further evaluation of epigenomics alterations is necessary, reflecting

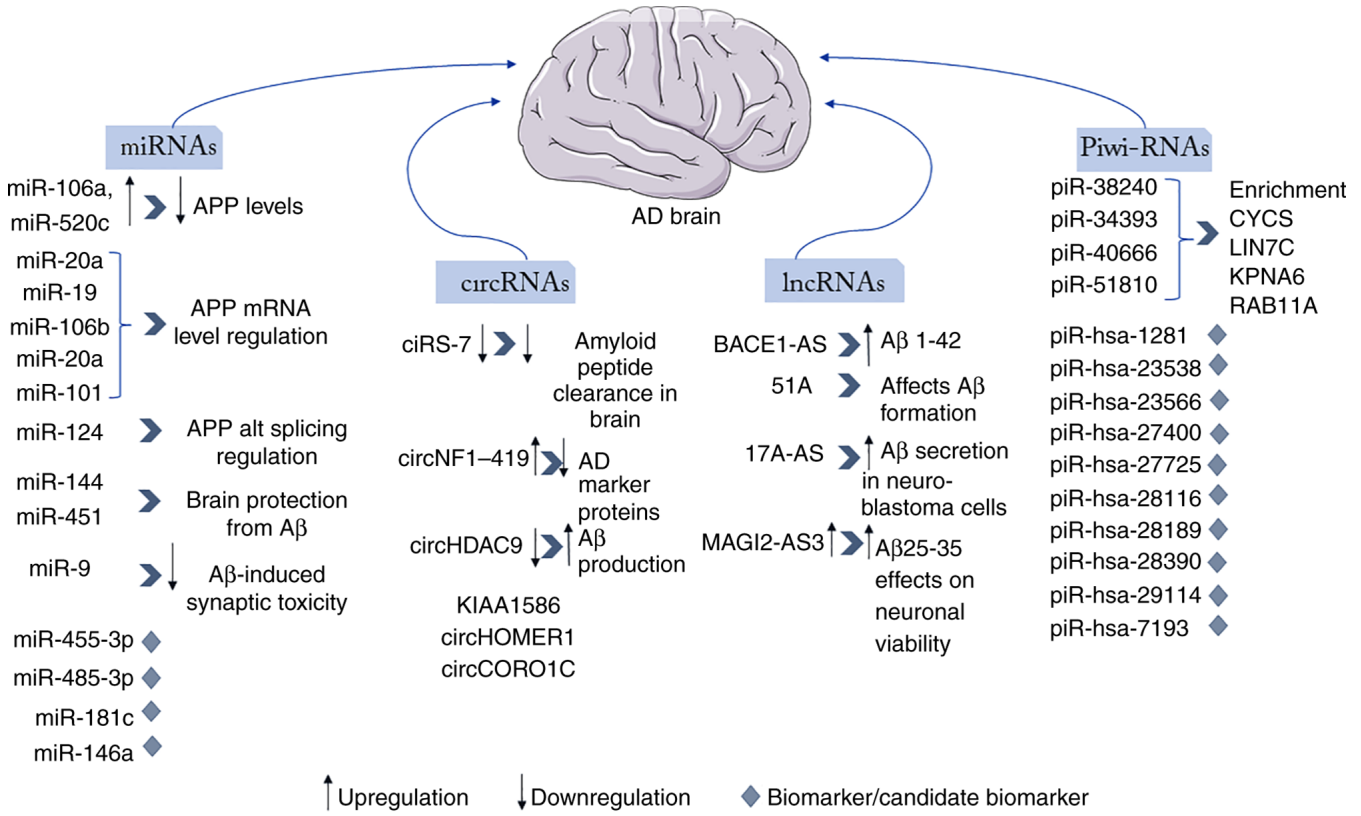

Figure 1. Illustration of the main ncRNAs of each category (miRNAs, circRNAs, piRNAs and lncRNAs) that are involved in AD and their mode of function and regulation in AD, including their upregulation or downregulation and the results of this alteration in their expression levels, as well their usage as biomarkers. ncRNAs, non-coding RNAs; miRNAs/miRs, microRNAs; circRNAs, circular RNAs; piRNAs, Piwi-interacting RNAs; lncRNAs, long non-coding RNAs; AD, Alzheimer's disease.

both changes in cell composition and cell-specific changes associated with AD pathology.

Transcriptomics. Transcriptome analysis provides the ability to evaluate the number of transcripts that result through alternative splicing, novel transcript identification and long and small ncRNAs. Several studies have highlighted the critical role of ncRNAs, mostly focusing on the role of miRNAs in AD (68,128-130). As aforementioned, miRNA profiles can be investigated in several biological fluids, including blood and CSF (131), as several AD-related miRNAs have been identified following an analysis of brain tissue in patients with AD (132). The analysis of circulating miRNAs is very promising for the study of the pathogenesis of AD; however, the heterogeneity of the research results requires the participation of a greater number of patients for the study of the transcriptome.

Proteomics. Proteomics studies provide the ability to discover and record potential biomarkers and validate potential candidate proteins in various diseases. Furthermore, mass spectrometry (MS) offers effective capabilities in the analysis and determination of proteins in combination with chromatographic or other separation techniques (133).

Through a standard proteomics platform, which includes two-dimensional gel electrophoresis in combination with MS, novel candidate biomarkers were identified in the biological fluids of patients with AD, primarily involved in the processing pathway of the Aβ peptide (134,135). Additionally, proteomics studies have identified eight protein biomarkers among 100 candidates, the levels of which tend to decrease in cases of AD (136).

A notable finding of extensive target proteomics studies is the presence of different isoforms of Aβ peptides, which arise through alternative pathways of APP degradation, identifying the isoforms Aβ42, Aβ40 and Aβ38, as well as APPα and APPβ in CSF and brain tissue samples from patients with AD (137-139). In addition, a significant increase in neurogranin levels in CSF was evidenced in patients with AD (140), in contrast to a significant decrease in ApoE levels in the serum of patients with AD (141).

Through a proteomics study, the interaction of brain transglutaminase interaction with APP, huntingtin and α-synuclein was observed, thus demonstrating the role of brain transglutaminase in the formation of protein aggregates in various neurodegenerative diseases (142). In addition, oxidatively modified proteins associated with tau and Aβ pathology have been identified in the brains of patients with AD through redox proteomics studies (143). Lastly, the study by Chiasserini *et al* (144) yielded information on 1,315 proteins, including neurodegenerative disease biomarkers such as APP, prion protein and DJ-1.

Metabolomics. Metabolomics studies examine and focus on metabolites, which are small molecules (<1,500 Da) that are involved in numerous biological functions and vary as a result of genetic, transcriptional and protein changes, as well

as environmental influences. The main techniques used in metabolomics studies are MS and nuclear magnetic resonance spectroscopy (28).

Significant alterations in the abundance of metabolites in the biological fluids of patients with AD have been observed. According to a previous study, a change in eight metabolites was detected, including acylcarnitine, sphingomyelins and glycerophospholipids, which were significantly increased in the CSF of patients (145). Furthermore, the quantitative analysis of 17 metabolites led to the observation of a significant increase in glycine and S-adenosylhomocysteine levels, with a concomitant decrease in the levels of S-adenosylmethionine in the CSF of patients diagnosed with AD (146). Similarly, shifts of 13 key metabolites were recorded at different stages of AD by researchers at the Alzheimer Disease Metabolomics Consortium, exhibiting associations with the CSF Aβ42 and t-tau/Aβ42 ratio, with cognitive function or brain atrophy (147). Finally, through two previous studies, 10 and 24 plasma lipids were detected to predict the conversion of healthy individuals to patients with AD (148,149).

Big data analysis. The development and analysis of a wealth of big data have been accompanied by advances in bioinformatics and computational programming (150,151). An example of a large-scale omics platform for AD is the Dominantly Inherited Alzheimer Network (DIAN) Central Archive (https://dian.wustl.edu/), which provides all the cognitive information, biomarkers and brain imaging information for AD, while allowing the data analysis of different domains and wide access to them (28).

The analysis and interpretation of big data are one of the most important issues of concern to the scientific community, with the main goal of analyzing the cross-platform association between data from different omics technologies. Furthermore, computational biology pipelines can aid the development of antibody-interacting drugs against neurodegenerative diseases, such as AD, a rapidly emerging field of increasing medicinal interest (152). Big data allow for rapid advances in personalized medicine; however, the heterogeneity and variability of omics data hinder the application of omics science (28).

Alterations in genomics, proteomics, epigenomics, transcriptomics, metabolomics and lipidomic levels may be associated with the development of neurodegenerative diseases, such as AD, worldwide. The development of computational tools for big data analysis based on phenotypic analytical prediction models remains a challenge for *de novo* drug design or effective drug repurposing (28). Deep learning methods can provide a potential solution to this challenge, allowing the exploitation of multi-omics data and helping to form an accurate representation of AD patients. This approach can allow researchers to develop effective personalized treatments and early diagnostic tools, as well as guide the design or repurposing of drugs in complex neurodegenerative pathologies (153). An integrative multi-omics approach, as previously described by Clark *et al* (154), yielded promising results, identifying novel molecular and pathway alterations which are related to the pathophysiological processes of AD. A notable strength of this approach is the identification of the main axes of inter-individual heterogeneity, critical for the development of tailored therapeutic interventions (154).

4. Conclusions and future perspectives

AD is a multifactorial disease, which in addition to the genetic and hereditary background, also occurs sporadically, due to epigenetic factors, which include ncRNAs. These molecules are implicated in a wide range of cellular processes and human diseases, including neurodegeneration. Several studies at various levels, including the molecular, cellular, physiological and epidemiological ones, have detected a rising number of ncRNAs involved in AD. As aforementioned, they participate in the three major pathogenic traits of AD that include the formation of Aβ plaques, the phosphorylation of tau, and the establishments of an inflammatory zone. In order to identify these ncRNAs, various studies have examined both cultured cell models and biological samples, such as brain, CSF and serum. In this context, their identification can be managed using sensitive methods of RNA analysis, including reverse transcription followed by conventional PCR or qPCR analyses for the survey of individual RNAs, or RNA-sequencing and analysis by microarray for the survey of larger panels of RNAs. These methods would be particularly useful if the diagnostic and prognostic ncRNAs were tested in tissues and fluids easily accessible, such as blood, urine and some epithelia (129).

In conjunction with these non-coding biomarkers, omics technologies are a promising tool for the study of AD pathology and patient variation, providing the ability to combine and correlate different types of data, the analysis of which could lead to personalized therapy and *de novo* development of more effective drugs. The integration of omics and clinical data and the development of novel experimental and computational strategies are essential in multifactorial diseases, such as AD. In these cases, high-throughput omics technologies can lead to the better understanding of the pathological changes in the brain, to the development of more accurate tools for the early diagnosis and prediction of the disease, to the development of new drugs, as well to the selection of the most beneficial and personalized therapies.

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Authors' contributions

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References

1. Cuyvers E and Sleegers K: Genetic variations underlying Alzheimer's disease: Evidence from genome-wide association studies and beyond. Lancet Neurol 15: 857-868, 2016.
2. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D and Jones E: Alzheimer's disease. Lancet 377: 1019-1031, 2011.
3. Braak H, Thal DR, Ghebremedhin E and Del Tredici K: Stages of the pathologic process in Alzheimer disease: Age categories from 1 to 100 years. J Neuropathol Exp Neurol 70: 960-969, 2011.
4. Fliss R, Le Gall D, Etcharry-Bouyx F, Chauviré V, Desgranges B and Allain P: Theory of Mind and social reserve: Alternative hypothesis of progressive Theory of Mind decay during different stages of Alzheimer's disease. Soc Neurosci 11: 409-423, 2016.
5. Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S and Van der Flier WM: Alzheimer's disease. Lancet 388: 505-517, 2016.
6. Krokidis MG, Exarchos TP and Vlamos P: Data-driven biomarker analysis using computational omics approaches to assess neurodegenerative disease progression. Math Biosci Eng 18: 1813-1832, 2021.
7. Verheijen J and Sleegers K: Understanding Alzheimer disease at the interface between genetics and transcriptomics. Trends Genetics 34: 434-447, 2018.
8. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W and Ferri CP: The global prevalence of dementia: A systematic review and metaanalysis. Alzheimers Dement 9: 63-75.e62, 2013.
9. Heppner FL, Ransohoff RM and Becher B: Immune attack: The role of inflammation in Alzheimer disease. Nat Rev Neurosci 16: 358-372, 2015.
10. Medeiros R, Kitazawa M, Passos GF, Baglietto-Vargas D, Cheng D, Cribbs DH and LaFerla FM: Aspirin-triggered lipoxin A4 stimulates alternative activation of microglia and reduces Alzheimer disease-like pathology in mice. Am J Pathol 182: 1780-1789, 2013.
11. Prokop S, Miller KR and Heppner FL: Microglia actions in Alzheimer's disease. Acta Neuropathol 126: 461-477, 2013.
12. Heneka MT, Kummer MP and Latz E: Innate immune activation in neurodegenerative disease. Nat Rev Immunol 14: 463-477, 2014.
13. Labzin LI, Heneka MT and Latz E: Innate Immunity and Neurodegeneration. Annu Rev Med 69: 437-449, 2018.
14. Aubry S, Shin W, Crary JF, Lefort R, Qureshi YH, Lefebvre C, Califano A and Shelanski ML: Assembly and interrogation of Alzheimer's disease genetic networks reveal novel regulators of progression. PLoS One 10: e0120352, 2015.
15. Chouraki V and Seshadri S: Genetics of Alzheimer's disease. Adv Genet 87: 245-294, 2014.
16. Greenough MA: The Role of presenilin in protein trafficking and degradation-implications for metal homeostasis. J Mol Neurosci 60: 289-297, 2016.
17. Millan MJ: Linking deregulation of non-coding RNA to the core pathophysiology of Alzheimer's disease: An integrative review. Prog Neurobiol 156: 1-68, 2017.
18. Huang YA, Zhou B, Wernig M and Südhof TC: ApoE2, ApoE3, and ApoE4 differentially stimulate APP transcription and Aβ secretion. Cell 168: 427-441.e21, 2017.
19. Jiang T, Yu JT, Tian Y and Tan L: Epidemiology and etiology of Alzheimer's disease: From genetic to non-genetic factors. Curr Alzheimer Res 10: 852-867, 2013.
20. Kanekiyo T, Xu H and Bu G: ApoE and Aβ in Alzheimer's disease: Accidental encounters or partners? Neuron 81: 740-754, 2014.
21. Vlachakis D, Papakonstantinou E, Sagar R, Bacopoulou F, Exarchos T, Kourouthanassis P, Karyotis V, Vlamos P, Lyketsos C, Avramopoulos D and Mahairaki V: Improving the utility of polygenic risk scores as a biomarker for Alzheimer's disease. Cells 10: 2021.
22. Yan Y, Zhao A, Qui Y, Li Y, Yan R, Wang Y, Xu W and Deng Y: Genetic Association of FERMT2, HLA-DRB1, CD2AP, and PTK2B Polymorphisms with Alzheimer's disease risk in the southern Chinese population. Front Aging Neurosci 12: 16, 2020.
23. Chin-Chan M, Navarro-Yepes J and Quintanilla-Vega B: Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. Front Cell Neurosci 9: 124, 2015.
24. Reitz C, Brayne C and Mayeux R: Epidemiology of Alzheimer disease. Nat Rev Neurol 7: 137-152, 2011.
25. Tremlett H, Bauer KC, Appel-Cresswell S, Finlay BB and Waubant E: The gut microbiome in human neurological disease: A review. Ann Neurol 81: 369-382, 2017.
26. Blennow K, Dubois B, Fagan AM, Lewczuk P, de Leon MJ and Hampel H: Clinical utility of cerebrospinal fluid biomarkers in the diagnosis of early Alzheimer's disease. Alzheimers Dement 11: 58-69, 2015.
27. Sancesario GM and Bernardini S: How many biomarkers to discriminate neurodegenerative dementia? Crit Rev Clin Lab Sci 52: 314-326, 2015.
28. Sancesario GM and Bernardini S: Alzheimer's disease in the omics era. Clin Biochem 59: 9-16, 2018.
29. Trushina E, Dutta T, Persson X-MT, Mielke MM and Petersen RC: Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. PLoS One 8: e63644, 2013.
30. Nday CM, Eleftheriadou D and Jackson G: Shared pathological pathways of Alzheimer's disease with specific comorbidities: Current perspectives and interventions. J Neurochem 144: 360-389, 2018.
31. Morgan SL, Naderi P, Koler K, Pita-Juarez Y, Prokopenko D, Vlachos IS, Tanzi RE, Bertram L and Hide WA: Most pathways can be related to the pathogenesis of Alzheimer's disease. Front Aging Neurosci 14: 846902, 2022.
32. Colpaert RMW and Calore M: Epigenetics and microRNAs in cardiovascular diseases. Genomics 113: 540-551, 2021.
33. Ramzan F, Vickers MH and Mithen RF: Epigenetics, microRNA and metabolic syndrome: A comprehensive review. Int J Mol Sci 22: 5047, 2021.
34. Suzuki H, Maruyama R, Yamamoto E and Kai M: Epigenetic alteration and microRNA dysregulation in cancer. Front Genet 4: 258, 2013.
35. Lau P, Bossers K, Janky R, Salta E, Frigerio CS, Barbash S, Rothman R, Sierksma AS, Thathiah A, Greenberg D, *et al*: Alteration of the microRNA network during the progression of Alzheimer's disease. EMBO Mol Med 5: 1613-1634, 2013.
36. Maoz R, Garfinkel BP and Soreq H: Alzheimer's disease and ncRNAs. Adv Exp Med Biol 978: 337-361, 2017.
37. Sun AX, Crabtree GR and Yoo AS: MicroRNAs: Regulators of neuronal fate. Curr Opin Cell Biol 25: 215-221, 2013.
38. Huang Y, Shen XJ, Zou Q, Wang SP, Tang SM and Zhang GZ: Biological functions of microRNAs: A review. J Physiol Biochem 67: 129-139, 2011.
39. Fiore R, Khudayberdiev S, Saba R and Schratt G: MicroRNA function in the nervous system. Prog Mol Biol Transl Sci 102: 47-100, 2011.

40. Goodall EF, Heath PR, Bandmann O, Kirby J and Shaw PJ: Neuronal dark matter: The emerging role of microRNAs in neurodegeneration. Front Cell Neurosci 7: 178, 2013.
41. Niwa R, Zhou F, Li C and Slack FJ: The expression of the Alzheimer's amyloid precursor protein-like gene is regulated by developmental timing microRNAs and their targets in Caenorhabditis elegans. Dev Biol 315: 418-425, 2008.
42. Patel N, Hoang D, Miller N, Ansaloni S, Huang Q, Rogers JT, Lee JC and Saunders AJ: MicroRNAs can regulate human APP levels. Mol Neurodegener 3: 10, 2008.
43. Fan X, Liu Y, Jiang J, Ma Z, Wu H, Liu T, Liu M, Li X and Tang H: miR-20a promotes proliferation and invasion by targeting APP in human ovarian cancer cells. Acta Biochim Biophys Sin (Shanghai) 42: 318-324, 2010.
44. Hébert SS, Horré K, Nicolaï L, Bergmans B, Papadopoulou AS, Delacourte A and De Strooper B: MicroRNA regulation of Alzheimer's Amyloid precursor protein expression. Neurobiol Dis 33: 422-428, 2009.
45. Vilardo E, Barbato C, Ciotti M, Cogoni C and Ruberti F: MicroRNA-101 regulates amyloid precursor protein expression in hippocampal neurons. J Biol Chem 285: 18344-18351, 2010.
46. Glinsky GV: An SNP-guided microRNA map of fifteen common human disorders identifies a consensus disease phenocode aiming at principal components of the nuclear import pathway. Cell Cycle 7: 2570-2583, 2008.
47. Delay C, Calon F, Mathews P and Hébert SS: Alzheimer-specific variants in the 3'UTR of Amyloid precursor protein affect microRNA function. Mol Neurodegener 6: 70, 2011.
48. Smith P, Al Hashimi A, Girard J, Delay C and Hébert SS: In vivo regulation of amyloid precursor protein neuronal splicing by microRNAs. J Neurochem 116: 240-247, 2011.
49. Kong Y, Wu J, Zhang D, Wan C and Yuan L: The role of miR-124 in drosophila Alzheimer's disease model by targeting delta in notch signaling pathway. Curr Mol Med 15: 980-989, 2015.
50. Schonrock N, Matamales M, Ittner LM and Götz J: MicroRNA networks surrounding APP and amyloid-β metabolism-implications for Alzheimer's disease. Exp Neurol 235: 447-454, 2012.
51. Rockenstein EM, McConlogue L, Tan H, Power M, Masliah E and Mucke L: Levels and alternative splicing of amyloid beta protein precursor (APP) transcripts in brains of APP transgenic mice and humans with Alzheimer's disease. J Biol Chem 270: 28257-28267, 1995.
52. Donev R, Newall A, Thome J and Sheer D: A role for SC35 and hnRNPA1 in the determination of amyloid precursor protein isoforms. Mol Psychiatry 12: 681-690, 2007.
53. Yang LB, Lindholm K, Yan R, Citron M, Xia W, Yang XL, Beach T, Sue L, Wong P, Price D, *et al*: Elevated beta-secretase expression and enzymatic activity detected in sporadic Alzheimer disease. Nat Med 9: 3-4, 2003.
54. Yang G, Song Y, Zhou X, Deng Y, Liu T, Weng G, Yu D and Pan S: MicroRNA-29c targets β-site amyloid precursor protein-cleaving enzyme 1 and has a neuroprotective role *in vitro* and *in vivo*. Mol Med Rep 12: 3081-3088, 2015.
55. Lei X, Lei L, Zhang Z, Zhang Z and Cheng Y: Downregulated miR-29c correlates with increased BACE1 expression in sporadic Alzheimer's disease. Int J Clin Exp Pathol 8: 1565-1574, 2015.
56. Zong Y, Wang H, Dong W, Quan X, Zhu H, Xu Y, Huang L, Ma C and Qin C: miR-29c regulates BACE1 protein expression. Brain Res 1395: 108-115, 2011.
57. Boissonneault V, Plante I, Rivest S and Provost P: MicroRNA-298 and microRNA-328 regulate expression of mouse beta-amyloid precursor protein-converting enzyme 1. J Biol Chem 284: 1971-1981, 2009.
58. Liu T, Huang Y, Chen J, Chi H, Yu Z, Wang J and Chen C: Attenuated ability of BACE1 to cleave the amyloid precursor protein via silencing long noncoding RNA BACE1-AS expression. Mol Med Rep 10: 1275-1281, 2014.
59. Zhang Y, Xing H, Guo S, Zheng Z, Wang H and Xu D: MicroRNA-135b has a neuroprotective role via targeting of β-site APP-cleaving enzyme 1. Exp Ther Med 12: 809-814, 2016.
60. Xie H, Zhao Y, Zhou Y, Wang D, Zhang S and Yang M: MiR-9 regulates the expression of BACE1 in dementia induced by chronic brain hypoperfusion in rats. Cell Physiol Biochem 42: 1213-1226, 2017.
61. Zhu HC, Wang LM, Wang M, Song B, Tan S, Teng JF and Duan DX: MicroRNA-195 downregulates Alzheimer's disease amyloid-β production by targeting BACE1. Brain Res Bull 88: 596-601, 2012.
62. Wang WX, Rajeev BW, Stromberg AJ, Ren N, Tang G, Huang Q, Rigoutsos I and Nelson PT: The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1. J Neurosci 28: 1213-1223, 2008.
63. Rybak-Wolf A and Plass M: RNA dynamics in Alzheimer's disease. Molecules 26: 5113, 2021.
64. Chang F, Zhang LH, Xu WP, Jing P and Zhan PY: microRNA-9 attenuates amyloidβ-induced synaptotoxicity by targeting calcium/calmodulin-dependent protein kinase kinase 2. Mol Med Rep 9: 1917-1922, 2014.
65. Janson J, Laedtke T, Parisi JE, O'Brien P, Petersen RC and Butler PC: Increased risk of type 2 diabetes in Alzheimer disease. Diabetes 53: 474-481, 2004.
66. Cheng C, Li W, Zhang Z, Yoshimura S, Hao Q, Zhang C and Wang Z: MicroRNA-144 is regulated by activator protein-1 (AP-1) and decreases expression of Alzheimer disease-related a disintegrin and metalloprotease 10 (ADAM10). J Biol Chem 288: 13748-13761, 2013.
67. Dubois B, Padovani A, Scheltens P, Rossi A and Dell'Agnello G: Timely diagnosis for Alzheimer's disease: A literature review on benefits and challenges. J Alzheimers Dis 49: 617-631, 2016.
68. Wei W, Wang ZY, Ma LN, Zhang TT, Cao Y and Li H: MicroRNAs in Alzheimer's disease: Function and potential applications as diagnostic biomarkers. Front Mol Neurosci 13: 2020.
69. Schwarzenbach H, Nishida N, Calin GA and Pantel K: Clinical relevance of circulating cell-free microRNAs in cancer. Nat Rev Clin Oncol 11: 145-156, 2014.
70. Bekris LM and Leverenz JB: The biomarker and therapeutic potential of miRNA in Alzheimer's disease. Neurodegener Dis Manag 5: 61-74, 2015.
71. Cogswell JP, Ward J, Taylor IA, Waters M, Shi Y, Cannon B, Kelnar K, Kemppainen J, Brown D, Chen C, *et al*: Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways. J Alzheimers Dis 14: 27-41, 2008.
72. Siedlecki-Wullich D, Català-Solsona J, Fábregas C, Hernández I, Clarimon J, Lleó A, Boada M, Saura CA, Rodríguez-Álvarez J and Miñano-Molina AJ: Altered microRNAs related to synaptic function as potential plasma biomarkers for Alzheimer's disease. Alzheimers Res Ther 11: 46, 2019.
73. Denk J, Boelmans K, Siegismund C, Lassner D, Arlt S and Jahn H: MicroRNA profiling of CSF reveals potential biomarkers to detect Alzheimer's disease. PLoS One 10: e0126423, 2015.
74. Alexandrov PN, Dua P and Lukiw WJ: Up-regulation of miRNA-146a in progressive, Age-related inflammatory neurodegenerative disorders of the human CNS. Front Neurol 5: 181, 2014.
75. Arena A, Iyer A, Milenkovic I, Kovacs GG, Ferrer I, Perluigi M and Aronica E: Developmental expression and dysregulation of miR-146a and miR-155 in Down's syndrome and mouse models of Down's syndrome and Alzheimer's disease. Curr Alzheimer Res 14: 1305-1317, 2017.
76. Kumar S and Reddy PH: Elevated levels of MicroRNA-455-3p in the cerebrospinal fluid of Alzheimer's patients: A potential biomarker for Alzheimer's disease. Biochim Biophys Acta Mol Basis Dis 1867: 166052, 2021.
77. Yu L, Li H, Liu W, Zhang L, Tian Q, Li H and Li M: MiR-485-3p serves as a biomarker and therapeutic target of Alzheimer's disease via regulating neuronal cell viability and neuroinflammation by targeting AKT3. Mol Genet Genomic Med 9: e1548, 2021.
78. Andreeva K and Cooper NGF: Circular RNAs: New players in gene regulation. Adv Bioscience Biotechnol 06No.06: 8, 2015.
79. Gruner H, Cortés-López M, Cooper DA, Bauer M and Miura P: CircRNA accumulation in the aging mouse brain. Sci Rep 6: 38907, 2016.
80. Akhter R: Circular RNA and Alzheimer's disease. Adv Exp Med Biol 1087: 239-243, 2018.
81. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK and Kjems J: Natural RNA circles function as efficient microRNA sponges. Nature 495: 384-388, 2013.
82. Lukiw WJ: Circular RNA (circRNA) in Alzheimer's disease (AD). Front Genet 4: 307, 2013.
83. Lonskaya I, Shekoyan AR, Hebron ML, Desforges N, Algarzae NK and Moussa CE: Diminished parkin solubility and Co-localization with intraneuronal amyloid-β are associated with autophagic defects in Alzheimer's disease. J Alzheimers Dis 33: 231-247, 2013.

84. Dube U, Del-Aguila JL, Li Z, Budde JP, Jiang S, Hsu S, Ibanez L, Fernandez MV, Farias F, Norton J, *et al*: An atlas of cortical circular RNA expression in Alzheimer disease brains demonstrates clinical and pathological associations. Nat Neurosci 22: 1903-1912, 2019.
85. Zhang Y, Yu F, Bao S and Sun J: Systematic characterization of circular RNA-associated CeRNA network identified novel circRNA biomarkers in Alzheimer's disease. Front Bioeng Biotechnol 7: 222, 2019.
86. Lu Y, Tan L and Wang X: Circular HDAC9/microRNA-138/Sirtuin-1 pathway mediates synaptic and amyloid precursor protein processing deficits in Alzheimer's disease. Neurosci Bull 35: 877-888, 2019.
87. Yang H, Wang H, Shang H, Chen X, Yang S, Qu Y, Ding J and Li X: Circular RNA circ_0000950 promotes neuron apoptosis, suppresses neurite outgrowth and elevates inflammatory cytokines levels via directly sponging miR-103 in Alzheimer's disease. Cell Cycle 18: 2197-2214, 2019.
88. Diling C, Yinrui G, Longkai Q, Xiaocui T, Yadi L, Xin Y, Guoyan H, Ou S, Tianqiao Y, Dongdong W, *et al*: Circular RNA NF1-419 enhances autophagy to ameliorate senile dementia by binding Dynamin-1 and Adaptor protein 2 B1 in AD-like mice. Aging (Albany NY) 11: 12002-12031, 2019.
89. Zhang M and Bian Z: The emerging role of circular RNAs in Alzheimer's disease and Parkinson's disease. Front Aging Neurosci 13: 691512, 2021.
90. Huang X and Wong G: An old weapon with a new function: PIWI-interacting RNAs in neurodegenerative diseases. Transl Neurodegener 10: 9, 2021.
91. Qiu W, Guo X, Lin X, Yang Q, Zhang W, Zhang Y, Zuo L, Zhu Y, Li CR, Ma C and Luo X: Transcriptome-wide piRNA profiling in human brains of Alzheimer's disease. Neurobiol Aging 57: 170-177, 2017.
92. Roy J, Sarkar A, Parida S, Ghosh Z and Mallick B: Small RNA sequencing revealed dysregulated piRNAs in Alzheimer's disease and their probable role in pathogenesis. Mol Biosyst 13: 565-576, 2017.
93. Mercer TR, Dinger ME and Mattick JS: Long non-coding RNAs: Insights into functions. Nat Rev Genet 10: 155-159, 2009.
94. Wilusz JE, Sunwoo H and Spector DL: Long noncoding RNAs: Functional surprises from the RNA world. Genes Dev 23: 1494-1504, 2009.
95. Clark MB and Mattick JS: Long noncoding RNAs in cell biology. Semin Cell Dev Biol 22: 366-376, 2011.
96. Martianov I, Ramadass A, Serra Barros A, Chow N and Akoulitchev A: Repression of the human dihydrofolate reductase gene by a non-coding interfering transcript. Nature 445: 666-670, 2007.
97. Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, Freier SM, Bennett CF, Sharma A, Bubulya PA, *et al*: The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. Mol Cell 39: 925-938, 2010.
98. Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, Kenzelmann-Broz D, Khalil AM, Zuk O, Amit I, Rabani M, *et al*: A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. Cell 142: 409-419, 2010.
99. Mourtada-Maarabouni M, Hedge VL, Kirkham L, Farzaneh F and Williams GT: Growth arrest in human T-cells is controlled by the non-coding RNA growth-arrest-specific transcript 5 (GAS5). J Cell Sci 121: 939-946, 2008.
100. Li F, Wang Y, Yang H, Xu Y, Zhou X, Zhang X, Xie Z and Bi J: The effect of BACE1-AS on β-amyloid generation by regulating BACE1 mRNA expression. BMC Mol Biol 20: 23, 2019.
101. Faghihi MA, Modarresi F, Khalil AM, Wood DE, Sahagan BG, Morgan TE, Finch CE, St Laurent G III, Kenny PJ and Wahlestedt C: Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase. Nat Med 14: 723-730, 2008.
102. Zeng T, Ni H, Yu Y, Zhang M, Wu M, Wang Q, Wang L, Xu S, Xu Z, Xu C, *et al*: BACE1-AS prevents BACE1 mRNA degradation through the sequestration of BACE1-targeting miRNAs. J Chem Neuroanat 98: 87-96, 2019.
103. Zhou Y, Ge Y, Liu Q, Li YX, Chao X, Guan JJ, Diwu YC and Zhang Q: LncRNA BACE1-AS promotes autophagy-mediated neuronal damage through the miR-214-3p/ATG5 signalling axis in Alzheimer's disease. Neuroscience 455: 52-64, 2021.
104. Parenti R, Paratore S, Torrisi A and Cavallaro S: A natural antisense transcript against Rad18, specifically expressed in neurons and upregulated during beta-amyloid-induced apoptosis. Eur J Neurosci 26: 2444-2457, 2007.
105. Li D, Zhang J, Li X, Chen Y, Yu F and Liu Q: Insights into lncRNAs in Alzheimer's disease mechanisms. RNA Biol 18: 1037-1047, 2021.
106. Ciarlo E, Massone S, Penna I, Nizzari M, Gigoni A, Dieci G, Russo C, Florio T, Cancedda R and Pagano A: An intronic ncRNA-dependent regulation of SORL1 expression affecting Aβ formation is upregulated in post-mortem Alzheimer's disease brain samples. Dis Model Mech 6: 424-433, 2013.
107. Massone S, Vassallo I, Fiorino G, Castelnuovo M, Barbieri F, Borghi R, Tabaton M, Robello M, Gatta E, Russo C, *et al*: 17A, a novel non-coding RNA, regulates GABA B alternative splicing and signaling in response to inflammatory stimuli and in Alzheimer disease. Neurobiol Dis 41: 308-317, 2011.
108. Zhang J and Wang R: Deregulated lncRNA MAGI2-AS3 in Alzheimer's disease attenuates amyloid-β induced neurotoxicity and neuroinflammation by sponging miR-374b-5p. Exp Gerontol 144: 111180, 2021.
109. Mus E, Hof PR and Tiedge H: Dendritic BC200 RNA in aging and in Alzheimer's disease. Proc Natl Acad Sci USA 104: 10679-10684, 2007.
110. Zhou X and Xu J: Identification of Alzheimer's disease-associated long noncoding RNAs. Neurobiol Aging 36: 2925-2931, 2015.
111. Magistri M, Velmeshev D, Makhmutova M and Faghihi MA: Transcriptomics profiling of Alzheimer's disease reveal neurovascular defects, altered Amyloid-β homeostasis, and deregulated expression of long noncoding RNAs. J Alzheimers Dis 48: 647-665, 2015.
112. Subramanian I, Verma S, Kumar S, Jere A and Anamika K: Multi-omics data integration, interpretation, and its application. Bioinformatics Biol Insights 14: 1177932219899051, 2020.
113. Peña-Bautista C, Baquero M, Vento M and Cháfer-Pericás C: Omics-based Biomarkers for the Early Alzheimer disease diagnosis and reliable therapeutic targets development. Curr Neuropharmacol 17: 630-647, 2019.
114. Tan MS, Cheah PL, Chin AV, Looi LM and Chang SW: A review on omics-based biomarkers discovery for Alzheimer's disease from the bioinformatics perspectives: Statistical approach vs machine learning approach. Comput Biol Med 139: 104947, 2021.
115. Giri M, Zhang M and Lü Y: Genes associated with Alzheimer's disease: An overview and current status. Clin Interv Aging 11: 665-681, 2016.
116. Ridge PG, Mukherjee S, Crane PK and Kauwe JSK; Alzheimer's Disease Genetics Consortium: Alzheimer's disease: Analyzing the missing heritability. PLoS One 8: e79771, 2013.
117. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, DeStafano AL, Bis JC, Beecham GW, Grenier-Boley B, *et al*: Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet 45: 1452-1458, 2013.
118. Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buros J, Gallins PJ, Buxbaum JD, Jarvik GP and Crane PK: Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet 43: 436-441, 2011.
119. Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, Bjornsson S, Huttenlocher J, Levey AI, Lah JJ, *et al*: Variant of TREM2 associated with the Risk of Alzheimer's disease. N Eng J Med 368: 107-116, 2012.
120. Cruchaga C, Karch CM, Jin SC, Benitez BA, Cai Y, Guerreiro R, Harari O, Norton J, Budde J, Bertelsen S, *et al*: Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease. Nature 505: 550-554, 2014.
121. Bennett DA, Yu L, Yang J, Srivastava GP, Aubin C and De Jager PL: Epigenomics of Alzheimer's disease. Transl Res 165: 200-220, 2015.
122. Lunnon K, Smith R, Hannon E, De Jager PL, Srivastava G, Volta M, Troakes C, Al-Sarraj S, Burrage J, Macdonald R, *et al*: Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. Nat Neurosci 17: 1164-1170, 2014.
123. De Jager PL, Srivastava G, Lunnon K, Burgess J, Schalkwyk LC, Yu L, Eaton ML, Keenan BT, Ernst J, McCabe C, *et al*: Alzheimer's disease: Early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. Nat Neurosci 17: 1156-1163, 2014.

124. Zhang K, Schrag M, Crofton A, Trivedi R, Vinters H and Kirsch W: Targeted proteomics for quantification of histone acetylation in Alzheimer's disease. Proteomics 12: 1261-1268, 2012.
125. Lu X, Deng Y, Yu D, Cao H, Wang L, Liu L, Yu C, Zhang Y, Guo X and Yu G: Histone acetyltransferase p300 mediates histone acetylation of PS1 and BACE1 in a cellular model of Alzheimer's disease. PLoS One 9: e103067, 2014.
126. Rao JS, Keleshian VL, Klein S and Rapoport SI: Epigenetic modifications in frontal cortex from Alzheimer's disease and bipolar disorder patients. Transl Psychiatry 2: e132, 2012.
127. Narayan PJ, Lill C, Faull R, Curtis MA and Dragunow M: Increased acetyl and total histone levels in post-mortem Alzheimer's disease brain. Neurobiol Dis 74: 281-294, 2015.
128. Zhang Y, Zhao Y, Ao X, Yu W, Zhang L, Wang Y and Chang W: The role of Non-coding RNAs in Alzheimer's disease: From regulated mechanism to therapeutic targets and diagnostic biomarkers. Front Aging Neurosci 13: 654978, 2021.
129. Idda ML, Munk R, Abdelmohsen K and Gorospe M: Noncoding RNAs in Alzheimer's disease. Wiley Interdiscip Rev RNA: Jan 12, 2018 (Epub ahead of print).
130. Wang M, Qin L and Tang B: MicroRNAs in Alzheimer's disease. Front Genet 10: 153, 2019.
131. Formosa A, Piro MC, Docimo R, Maturo P, Sollecito DR, Kalimutho M, Sancesario G, Barlattani A, Melino G, Candi E and Bernardini S: Salivary miRNAome profiling uncovers epithelial and proliferative miRNAs with differential expression across dentition stages. Cell Cycle 10: 3359-3368, 2011.
132. Dehghani R, Rahmani F and Rezaei N: MicroRNA in Alzheimer's disease revisited: Implications for major neuropathological mechanisms. Rev Neurosci 29: 161-182, 2018.
133. Shevchenko G, Konzer A, Musunuri S and Bergquist J: Neuroproteomics tools in clinical practice. Biochim Biophys Acta 1854: 705-717, 2015.
134. Henkel AW, Müller K, Lewczuk P, Müller T, Marcus K, Kornhuber J and Wiltfang J: Multidimensional plasma protein separation technique for identification of potential Alzheimer's disease plasma biomarkers: A pilot study. J Neural Transm (Vienna) 119: 779-788, 2012.
135. Thambisetty M, Simmons A, Velayudhan L, Hye A, Campbell J, Zhang Y, Wahlund LO, Westman E, Kinsey A, Güntert A, *et al*: Association of plasma clusterin concentration with severity, pathology, and progression in Alzheimer disease. Arch Gen Psychiatry 67: 739-748, 2010.
136. Korolainen MA, Nyman TA, Aittokallio T and Pirttilä T: An update on clinical proteomics in Alzheimer's research. J Neurochem 112: 1386-1414, 2010.
137. Pannee J, Portelius E, Oppermann M, Atkins A, Hornshaw M, Zegers I, Höjrup P, Minthon L, Hansson O, Zetterberg H, *et al*: A selected reaction monitoring (SRM)-based method for absolute quantification of Aβ 38, Aβ 40, and Aβ 42 in cerebrospinal fluid of Alzheimer's disease patients and healthy controls. J Alzheimers Dis 33: 1021-1032, 2013.
138. Erik P, Niklas M, Ulf A, Kaj B and Henrik Z: Novel AβIsoforms in Alzheimer's disease-their role in diagnosis and treatment. Curr Pharmaceutical Design 17: 2594-2602, 2011.
139. Brinkmalm G, Brinkmalm A, Bourgeois P, Persson R, Hansson O, Portelius E, Mercken M, Andreasson U, Parent S, Lipari F, *et al*: Soluble amyloid precursor protein α and β in CSF in Alzheimer's disease. Brain Res 1513: 117-126, 2013.
140. Thorsell A, Bjerke M, Gobom J, Brunhage E, Vanmechelen E, Andreasen N, Hansson O, Minthon L, Zetterberg H and Blennow K: Neurogranin in cerebrospinal fluid as a marker of synaptic degeneration in Alzheimer's disease. Brain Res 1362: 13-22, 2010.
141. Han SH, Kim JS, Lee Y, Choi H, Kim JW, Na DL, Yang EG, Yu MH, Hwang D, Lee C and Mook-Jung I: Both targeted mass spectrometry and flow sorting analysis methods detected the decreased serum apolipoprotein E level in Alzheimer's disease patients. Mol Cell Proteomics 13: 407-419, 2014.
142. André W, Nondier I, Valensi M, Guillonneau F, Federici C, Hoffner G and Djian P: Identification of brain substrates of transglutaminase by functional proteomics supports its role in neurodegenerative diseases. Neurobiol Dis 101: 40-58, 2017.
143. Sultana R, Perluigi M and Butterfield DA: Lipid peroxidation triggers neurodegeneration: A redox proteomics view into the Alzheimer disease brain. Free Radic Biol Med 62: 157-169, 2013.
144. Chiasserini D, van Weering JRT, Piersma SR, Pham TV, Malekzadeh A, Teunissen CE, de Wit H and Jiménez CR: Proteomic analysis of cerebrospinal fluid extracellular vesicles: A comprehensive dataset. J Proteomics 106: 191-204, 2014.
145. Koal T, Klavins K, Seppi D, Kemmler G and Humpel C: Sphingomyelin SM(d18:1/18:0) is significantly enhanced in cerebrospinal fluid samples dichotomized by pathological amyloid-β42, tau, and phospho-tau-181 levels. J Alzheimers Dis 44: 1193-1201, 2015.
146. Guiraud SP, Montoliu I, Da Silva L, Dayon L, Galindo AN, Corthésy J, Kussmann M and Martin FP: High-throughput and simultaneous quantitative analysis of homocysteine-methionine cycle metabolites and co-factors in blood plasma and cerebrospinal fluid by isotope dilution LC-MS/MS. Anal Bioanal Chem 409: 295-305, 2017.
147. Toledo JB, Arnold M, Kastenmüller G, Chang R, Baillie RA, Han X, Thambisetty M, Tenenbaum JD, Suhre K, Thompson JW, *et al*: Metabolic network failures in Alzheimer's disease: A biochemical road map. Alzheimers Dement 13: 965-984, 2017.
148. Mapstone M, Cheema AK, Fiandaca MS, Zhong X, Mhyre TR, MacArthur LH, Hall WJ, Fisher SG, Peterson DR, Haley JM, *et al*: Plasma phospholipids identify antecedent memory impairment in older adults. Nat Med 20: 415-418, 2014.
149. Fiandaca MS, Zhong X, Cheema AK, Orquiza MH, Chidambaram S, Tan MT, Gresenz CR, FitzGerald KT, Nalls MA, Singleton AB, *et al*: Plasma 24-metabolite panel predicts preclinical transition to clinical stages of Alzheimer's disease. Front Neurol 6: 237, 2015.
150. Pimplikar SW: Multi-omics and Alzheimer's disease: A slower but surer path to an efficacious therapy? Am J Physiol Cell Physiol 313: C1-C2, 2017.
151. Moreno-Indias I, Lahti L, Nedyalkova M, Elbere I, Roshchupkin G, Adilovic M, Aydemir O, Bakir-Gungor B, Santa Pau EC, D'Elia D, *et al*: Statistical and machine learning techniques in human microbiome studies: Contemporary challenges and solutions. Front Microbiol 12: 635781, 2021.
152. Papageorgiou L, Papakonstantinou E, Salis C, Polychronidou E, Hagidimitriou M, Maroulis D, Eliopoulos E and Vlachakis D: Drugena: A fully automated immunoinformatics platform for the design of antibody-drug conjugates against neurodegenerative diseases. Adv Exp Med Biol 1194: 203-215, 2020.
153. Termine A, Fabrizio C, Strafella C, Caputo V, Petrosini L, Caltagirone C, Giardina E and Cascella R: Multi-Layer picture of neurodegenerative diseases: Lessons from the use of big data through artificial intelligence. J Pers Med 11: 280, 2021.
154. Clark C, Dayon L, Masoodi M, Bowman GL and Popp J: An integrative multi-omics approach reveals new central nervous system pathway alterations in Alzheimer's disease. Alzheimers Res Ther 13: 71, 2021.