REVIEW ARTICLE

Omics-based Biomarkers for the Early Alzheimer Disease Diagnosis and Reliable Therapeutic Targets Development

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Abstract: Background: Alzheimer's disease (AD), the most common cause of dementia in adulthood, has great medical, social, and economic impact worldwide. Available treatments result in symptomatic relief, and most of them are indicated from the early stages of the disease. Therefore, there is an increasing body of research developing accurate and early diagnoses, as well as disease-modifying therapies.

Objective: Advancing the knowledge of AD physiopathological mechanisms, improving early diagnosis and developing effective treatments from omics-based biomarkers.

Methods: Studies using omics technologies to detect early AD, were reviewed with a particular focus on the metabolites/lipids, micro-RNAs and proteins, which are identified as potential biomarkers in non-invasive samples.

Results: This review summarizes recent research on metabolomics/lipidomics, epigenomics and proteomics, applied to early AD detection. Main research lines are the study of metabolites from pathways, such as lipid, amino acid and neurotransmitter metabolisms, cholesterol biosynthesis, and Krebs and urea cycles. In addition, some microRNAs and proteins (microglobulins, interleukins), related to a common network with amyloid precursor protein and tau, have been also identified as potential biomarkers. Nevertheless, the reproducibility of results among studies is not good enough and a standard methodological approach is needed in order to obtain accurate information.

Conclusion: The assessment of metabolomic/lipidomic, epigenomic and proteomic changes associated with AD to identify early biomarkers in non-invasive samples from well-defined participants groups will potentially allow the advancement in the early diagnosis and improvement of therapeutic interventions.

Keywords: Metabolomics, lipidomics, epigenomics, proteomics, Alzheimer disease, biomarkers, mild cognitive impairment.

1. INTRODUCTION

Alzheimer's disease (AD), clinically characterized by the progressive impairment of memory, and cognitive, behavioural and functional abilities, has great public health, social, and economic impacts since it is the most common form of dementia and the principal cause of adult disability. The growing number of patients and mortality and morbidity associated to AD is becoming a major concern in countries with aging populations [1]. In general, the incidence level of AD after 70 years of age reaches 9% of the population [2], involving a significant increase in medical and social costs

[3]. In addition, taking into account the demographic change, it is estimated that in 2030, approximately 56.5 million people will suffer from AD in the world [4]. Nowadays, available treatments only achieve a symptomatic relief, and most of them are indicated at early stages of the dementia [5]. In this sense, some biomarkers of AD pathology and physiopathology improve the *in vivo* diagnosis and their use is modifying the classic concept of this entity. Actually, research about early and minimally invasive AD biomarkers, as well as potential disease-treatment therapies using omics techniques were reviewed in this work.

1.1. Current Diagnosis of Alzheimer's Disease

From a clinical point of view, AD is a pathological condition characterized by specific structural changes in the brain and a characteristic pattern of cognitive and functional

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abilities. Briefly, its symptomatic development consists of three phases: i) preclinical phase, characterized by a normal cognitive status while ongoing brain pathology is being generated; ii) Mild cognitive impairment (MCI), characterized by the presence of symptoms and signs of cognitive deficit secondary to fully developed brain pathology. The habitual performance on daily life activities, however, is not altered; and iii) dementia, characterized by progressively greater cognitive impairment affecting the ability of carrying out every day's activities [6]. Cognitive markers are altered at the MCI phase, while image and cerebrospinal fluid (CSF) markers start to get alter from the preclinical phase [7]. Current research diagnostic criteria from the National Institute on Aging and the Alzheimer's Association (NIA-AA) propose the simultaneous use of neuropsychological evaluations, neuroimaging techniques, and biomarkers in CSF samples in order to obtain a reliable and early AD diagnosis [8, 9]. In this sense, the standard diagnosis of MCI due to AD is based on global neuropsychological evaluations (Clinical Dementia Rating, CDR [10]; Global Deterioration Scale, GDS [11]), specific cognitive evaluations (episodic memory, attention, language, recognition, praxis, executive function), structural and functional neuroimaging (Magnetic Resonance Imaging, MRI; positron emission tomography, PET) [12], and CSF biomarkers (β -amyloid, total tau (t-tau), phosphorylated tau (p-tau)) (Table 1). Currently, AD diagnostic criteria allow *in vivo* diagnosis by means of pathological processes detection; however, they show some limitations to be introduced in clinical practice. In fact, MRI features are relatively not AD specific or sensitive, PET is a very expensive imaging procedure not available in most hospitals, CSF samples are obtained by an invasive procedure with some contraindications and secondary effects, so it is commonly rejected by patients, and neuropsychological evaluations are time-consuming [4, 5]. A non-invasive and non-expensive diagnostic method is required in the AD research field and in the global dementia assistance network to improve treatment and

prognosis management. In the searching for specific and reliable AD biomarkers in non-invasive biological samples, the omics technologies play an important role since they can address the complex diagnosis from different molecular levels.

1.2. Physiopathological Mechanisms of Alzheimer's Disease

95% cases of AD are late-onset and sporadic, while around 5% of AD cases are early-onset and associated to genetic mutations in some proteins (*e.g.* presenilin 1, presenilin 2, amyloid precursor protein), which is known as familial AD. Interestingly, biochemical changes produced in familial AD seem similar to those in sporadic AD [13]. In general, the physiopathology of AD is characterized by a loss of synapses [14], mainly related to the extracellular deposition of the β -amyloid peptide in the form of senile plaques, one of the classic histological marks of AD. This β -amyloid substance comes from the cleavage of the amyloid precursor protein (APP) by the beta-site APP-cleaving enzyme 1 (BACE1) [15]. Another histological mark of the disease is tau protein intracellular accumulation. This protein is involved in the maintenance of the cellular cytoskeleton microtubule network, and its function is enzymatically regulated by different phosphorylation degree. In fact, this hyperphosphorylated protein is less functional and forms oligomers that tend to autoaggregate and sedimentate, resulting in the formation of neurofibrillary tangles [16]. Also, chronic inflammation could be an important physiopathological mechanism, contributing to the metabolism and accumulation of β -amyloid peptide [17]. Both amyloid and tau pathologies usually spread from medial temporal lobe grey matter to the rest of cortical grey matter in a relatively predictable pattern. Initial involvement in medial temporal lobe structures that are involved in the correct episodic memory working, explains the memory impairment as the first disease symptom. Nevertheless, variations in pathology spreading would explain the different damage degrees in brain cor-

Table 1. Standard criteria for Alzheimer Disease diagnosis.

Clinical Markers		Classification of Patients		
		MCI not Due to AD	MCI Due to AD [8]	Mild-moderate Dementia Due to AD [9]
Global evaluation tests	CDR [10]	0.5	0.5	0.5-2
	GDS [11]	3	3	4-6
Functionality evaluation test	Normal	Normal	Impaired	
Neuroimaging	Structural (NMR-TAC)	Normal	Medial temporal atrophy (normal-mild degree)	Medial temporal atrophy (moderated degree)
	Functional (PET-TAC)	Normal	Normal or parietotemporal hypometabolism	Parietotemporal hypometabolism
		- amyloid PET	-/+ amyloid PET	+ amyloid PET
CSF t-tau (mg dL ⁻¹) ^s		<400	>400	>400
CSF p-tau (mg dL ⁻¹)		<70	>70	>70
CSF β -amyloid (mg dL ⁻¹) ^s		>700	<700	<700

*SD (Standard deviation). ^sThese cut-off values depend on the laboratory and measurement methodology.

Fig. (1). Diagram representing the multi-omic approach to identify early AD biomarkers in non-invasive samples, and to develop new therapeutic targets. (The color version of the figure is available in the electronic copy of the article).

tex among patients, involving in some cases also language disturbance, frontal lobe dysfunction, and even agnostic or apraxia syndromes. Moreover, advanced-age patients show concurrent brain comorbidities (e.g. depression, psychiatric disorders...). However, the prevalent vision of the physiopathological mechanisms of AD is considered incomplete, and it could be the cause of inability to develop effective therapeutic targets based on the AD molecular pathogenesis.

Recent studies suggest that MCI due to AD is the result of an imbalance in the interactions among different brain cells types, pathogenic forms of tau and amyloid proteins, and the brain signaling pathways impairment [18]. In this way, the neurodegenerative process would affect each cell type at multiple levels (epigenomic, transcriptomic, metabolomic/lipidomic, proteomic). Therefore, a complete knowledge of the AD mechanisms could be achieved from a multi-omic approach applied to different biological samples. In this sense, the omic tools would contribute importantly to the knowledge of the early AD physiopathological mechanisms and develop specific and reliable AD biomarkers in common biological samples.

1.3. Omic Sciences and Alzheimer's Disease

The development of omic platforms and advances in bioinformatics are generating a large volume of data from pa-

tients with AD and healthy people, at the same age [19]. Therefore, in the next few years, the omic studies will allow a relevant advance in the knowledge of AD at multiple levels: i) Identification of biomarkers to be used in the diagnosis or prognosis of the disease; ii) Advance in the knowledge of possible physiopathological mechanisms; iii) Development of new and effective therapeutic strategies. Metabolomics, epigenomics and proteomics rank among the most widely employed omic tools in clinical studies. Fig. 1 depicts a multi-omic approach to identify different-nature biomarkers and to develop a reliable diagnosis model, as well as potential therapeutic targets, taking into account the complex physiopathology of AD. Of note, the potential use of omics techniques in the development of early AD biomarkers is very relevant, since small changes at molecular or genomic levels can be detected with high specificity and sensitivity several years before the clinical characteristics appear. However, nowadays CSF biomarkers and imaging-based technologies are quite good at diagnosis once the disease has been established.

1.3.1. Metabolomics

Metabolomics reflects changes in the metabolome representing a precise biochemical phenotype of the organism in health and disease that can be easily applied to different biofluids [20]. This allows a reliable approach to the com-

Fig. (2). Flow diagram representing the search, screening and final selection of the studies included in this review.

plex AD nature in the efficient development of a therapeutic strategy. Of note, only a few studies have applied non-targeted approaches to identify global changes in metabolites and metabolic pathways in AD [21]. Among these, some studies in plasma have identified several pathways affected in AD (biosynthesis and metabolism of amino acids, cholesterol, lipids, neurotransmitters, urea cycle and Krebs cycle) [22], highlighting that lipid dysfunction plays an important role in AD physiopathology [23].

1.3.2. Epigenomics

Epigenomics is an interesting approach to the knowledge of the physiopathology of AD since the expression patterns of certain genes implicated in the development of the disease (*APP*, *PSEN1*, *PSEN2*, *BACE1*), as well as secretase enzymes and inflammatory response, are altered by epigenetic modifications (DNA methylation [24], expression of non-coding genes transcripts (microRNAs [25])). These impairments lead to the deposition of β -amyloid plaques. Currently, there are few studies that evaluate the diagnostic capacity of epigenetic changes in peripheral fluids from AD patients [26]. However, next generation sequencing (NGS) technology is presented as a viable approach to carry it out [27].

1.3.3. Proteomics

The proteomic studies in biological fluids of AD patients are promising, since there is a molecular exchange between the brain and these fluids. Specifically, the brain uses signaling proteins, found in blood, to control bodily functions (*e.g.* peripheral and central inflammatory, and immune mecha-

nisms) [28]. Therefore, changes in these signaling proteins probably cause a specific phenotype of AD in blood samples [29]. In this sense, proteomics also constitutes an important approach to the development of efficient therapeutic targets. Recent studies in plasma have identified characteristic proteomic signatures of AD [30], although it is necessary to explore the reproducibility of these results.

2. MATERIALS AND METHODS

Data search was completed on March 29th 2018. Literature references were obtained after a systematic searching of PubMed, ISI Web of Science, and ScienceDirect databases. Keywords used in databases were: “Alzheimer Disease” AND “Mild cognitive impairment” AND “Metabolomics or Epigenomics or Proteomics” AND “biomarker”. Studies in English with human subjects, carried out in non-invasive samples and in the last 5 years (2013-2018) were considered for inclusion. Data search was supplemented with the reference lists from all the studies included. In Fig. 2 we can see the flow diagram of the selected articles.

3. RESULTS AND DISCUSSION

Several studies using omic technologies to identify early AD biomarkers in non-invasive biological samples have been revised. Most of these studies have been carried out in patients diagnosed with MCI or AD by means of classic Petersen or National Institute of Neurological and Communicative Disorders and Stroke, and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria (neuropsychological evaluation, neuroimaging-MRI)

[31, 32], so using unspecific clinical-based criteria. Use of obsolete criteria defining a pathologic group of patients highly contrasts with the intended high-performance omic technologies, which have the advantage of identifying small changes at molecular level even before the first clinical manifestations appear. In this sense, omics technologies potentially constitute a relevant tool in the development of early AD biomarkers in asymptomatic individuals. The reviewed studies have been classified as metabolomics/lipidomics, epigenomics and proteomics studies, and their corresponding biomarkers (metabolites/lipids, proteins, microRNAs), type of sample and results are summarized in Table 2. As we can see, most of them developed metabolomics or proteomics analysis, the participants were classified into 3 groups (AD, MCI, and healthy control (HC)), and the sample types most used were plasma and serum. In general, these omic tools identified lipids, aminoacids, and nucleic acids metabolisms as early AD impaired pathways [22], as well as deregulated microRNAs and proteins [26, 30], potentially providing an important advance in the knowledge of AD mechanisms; also, different types of biomarkers (metabolites/lipids, microRNAs, proteins) were determined, evaluating their specificity and accuracy in AD diagnosis.

3.1. Metabolites

Metabolic alterations triggered by the development of AD involve changes in the concentration of different metabolites, providing information about its physiopathology. In Table 2, we can see that metabolites related to oxidative stress (isoprostanes, nitro-fatty acids, oxidized lipoproteins) and inflammation, as well as Krebs and urea cycle metabolic pathways differ between AD cases and controls [33-37]. In addition, glutamine homeostasis [33], energy metabolism [34], and nucleic acid and amine metabolisms are described as altered already in early AD stages [35, 36, 38], and higher levels of glutamic acid, taurine, and hypoxanthine were associated with greater risk of dementia [40]. A recent study showed high levels of biomarkers related to hypoxia (glutamic acid, 2,4-dihydroxybutanoic acid) in serum from patients at early stages of AD [41], but no significant differences were observed between AD and other brain disorders.

In general, most of the metabolites identified as potential biomarkers are related to lipid and amino acid metabolisms (Table 2) [22, 39, 42]. Actually, multiple aminoacids (glutamine, glutamic acid, tyrosine) showed decreased levels in AD [38, 37], especially those related to lysine and tyrosine pathways, as well as tryptophan pathway [40, 43]. In this sense, tyrosine is the precursor of neurotransmitters dopamine and norepinephrine, which play an important role in cognitive control; while tryptophan is the precursor of neurotransmitter serotonin and the hormone melatonin. However, some studies showed increased levels in AD or no differences between participants' groups for some aminoacids [22, 35-38, 40, 41]. As regards the lipid metabolism, significant differences in the levels of some compounds were observed between early AD and HC groups (Table 2). Actually, plasma levels of sphingolipids and sphingomyelins showed statistically significant difference between AD and HC patients [23, 44], even in preclinical AD stages [39]. Specifically, lower concentrations of phosphatidylcholines (PC)

were found in AD plasma and in soluble lysates of platelets [42, 45, 46]. According to their predictive ability, sphingomyelin and PCs [42], as well as other phospholipids that were altered in MCI and dementia, showed satisfactory predictive indexes [39, 41, 42]. However, discrepant trends were observed for these metabolites in different studies [44, 47]. In addition, fatty acids [22, 45, 48], cholesteryl esters [35, 49, 50], and triglycerides have also been found highly reliable in differentiating MCI-AD from HC participants and led to the development of novel and reliable tests [49]. Specifically, low levels of triglycerides were found in AD patients [37], this agrees with some recent lipidomics studies reporting a decrease in most of the plasma lipids in AD [23, 38, 51], probably it is associated to neural hypometabolism. On the other hand, inconsistent results were observed for ceramides [23, 38, 52] and acylcarnitines levels in plasma [33, 34, 38, 39, 51, 53], obtaining not satisfactory conclusions about the energy metabolism involvement in AD; while some diacylglycerols were elevated in MCI and AD [38, 54], constituting potential biomarkers for AD diagnosis. Nevertheless, the reproducibility of the studies' results is very low. It can be explained by the high heterogeneity of clinical cohorts (stable or progressive MCI, mild or moderate disease severity, probable AD) [51, 52, 54], as well as by the impairment grade of each metabolic pathway in different stages of the disease. In addition, few studies employed a CSF biomarker-confirmed diagnosis [37], so metabolic alterations found in AD patients could not be disease-specific biochemical changes [51]. Other studies include a limited number of participants and so they are more easily affected by selection bias or random associations, as well as by the lack of reproducibility across clinical cohorts [42, 46, 48, 51].

From previously identified metabolites showing differences between MCI-AD and HC participants, some metabolomics studies have generated panels of biomarkers [22], and diagnostic models using logistic regression [55] or partial least squares - discriminant analysis (PLS-DA) [35] that describe the sensitivity and accuracy of the biomarker panel [33]. According to this, satisfactory accuracy was obtained from cholesteryl esters selected by a random forest approach (Area under curve receiver operating characteristic (AUC-ROC) 0.792, 82% sensitivity, 77% specificity) [50], as well as from phospholipids/metabolites (AUC-ROC 0.607) [47], and urea cycle metabolites (AUC-ROC 0.97) [36]. In another final model that accurately distinguished MCI-AD from HC participants (AUC-ROC 0.826) [38], the individual discriminatory power of each included metabolite was evaluated, observing that glutamic acid and aspartic acid were the most informative biomarkers (AUC-ROC 0.677 and 0.676, respectively). However, most of the studies did not evaluate the individual diagnostic performance of potential biomarkers [36, 48, 54], since single metabolites usually were poor predictors, showing low specificity and selectivity to discriminate between MCI-AD and HC subjects [51]. Regarding metabolites specificity among diseases, no differences were found for 2,4-dihydroxybutanoic acid (hypoxia metabolite) across AD, normal pressure hydrocephalus and brain tumour samples [41], as well as for phosphatidylcholines in AD and non-demented older individuals [46]. So, metabolomics is considered a useful tool to identify early

Table 2. Early and non-invasive biomarkers obtained from omics techniques and found in AD studies in literature.

Refs.	Participants	Omics Technique	Biomarker	Sample	Results
[22]	AD (n=57), MCI (n=58), HC (n=57)	Metabolomics	Arachidonic acid, N,N-dimethylglycine, thymine, glutamine, glutamic acid, cytidine, 2-aminoadipic acid, N,N-dimethylglycine, 5,8-tetradecadienoic acid.	plasma	Thymine ↑, arachidonic acid ↓, 2-aminoadipic acid ↑, glutamine ↑, proline ↑, N,N-dimethylglycine ↓, and 5,8-tetradecadienoic acid ↓ in AD. A panel from these metabolites was able to differentiate MCI patients from HC. Metabolisms involved: fatty acid, one-carbon, amino acid, nucleic acid.
[33]	AD (n=75), MCI (n=17), HC (n=45)	Metabolomics	Acylcarnitines, oleamide, monoglycerides, phenylacetylglutamine.	serum	Mitochondrial dysfunction, alterations in glutamine homeostasis in AD (Acylcarnitines ↑ in AD in relation to mitochondrial dysfunction, oleamide ↓ and monoglycerides ↓ as a result of defects in endocannabinoid system, phenylacetylglutamine ↑ in AD).
[34]	AD (n=42), MCI (n=14), HC (n=37)	Metabolomics	Choline, creatinine, asymmetric dimethyl-arginine, homocysteine, cysteine disulfide, phenylalanyl-phenylalanine, acylcarnitines, asparagine, methionine, histidine, carnitine, acetyl-spermidine.	serum	Oxidative stress and energy metabolism are pathways involved in AD (Choline ↑, creatinine ↑, asymmetric dimethyl-arginine ↑, homocysteine ↑, cysteine disulfide ↑, phenylalanyl-phenylalanine ↑, acylcarnitines ↑, asparagine ↓, methionine ↓, histidine ↓, carnitine ↓, acetyl-spermidine ↓ in AD).
[35]	MCI (n=16), MCI-AD (n = 19), HC (n = 37)	Metabolomics	Polyamines, L-arginine	plasma	Cholesterol ↑, glucose ↑, prostaglandine ↑, aminoacids, achieving a OPLS-DA statistical model (R ² = 99.1%; Q ² = 97%). Specifically, a multivariate model predicted cases of MCI with 97.6% accuracy.
[36]	superior memory (n = 41), normal memory (n = 109), MCI-AD (n = 74)	Metabolomics	Aspartate, Hydroxyhexadecadienylcarnitine, 3-Hydroxypalmitoleylcarnitine, Arginine, Valerylarginine, Asparagine, Citrulline, Nitrotyrosine, Histamine.	plasma	Aspartate ↓, Hydroxyhexadecadienylcarnitine, 3-Hydroxypalmitoleylcarnitine, Arginine ↑, Valerylarginine, Asparagine ↑, Citrulline ↓, Nitrotyrosine ↑, Histamine ↑ in AD. Urea cycle metabolites allowed to develop a predictive regression model with ROC AUC of 0.97 [95% CI: 0.92 – 1.0].
[37]	AD (n=127), HC (n=121)	Metabolomics	Tyrosine, glycylglycine, glutamine, lysophosphatic acid C18:2, platelet-activating factor C16:0.	plasma	26 metabolites were differentially expressed. Network models identified five hubs of metabolic deregulation: tyrosine ↓, glycylglycine ↓, glutamine ↓, lysophosphatic acid C18:2, TGs ↓ in AD, and platelet-activating factor C16:0.
[38]	AD (n=100), MCI (n=58), HC (n=93)	Metabolomics	Aminoacids (glutamic acid, alanine, aspartic acid), one non-esterified fatty acid (22:6n-3,DHA), one bile acid (deoxycholic acid), one phosphatidylethanolamine [PE(36:4)], one sphingomyelin [SM(39:1)].	plasma	DHA ↓, sphingomyelin ↓, ceramides ↓, TG ↓, glutamic acid ↓, aspartic acid ↓, alanine ↑, acylcarnitines ↑ in AD. The final model accurately distinguished AD from HC patients (AUC, 0.918). Importantly, the model also distinguished MCI from HC patients (AUC, 0.826), indicating its potential diagnostic utility in early disease stages.
[39]	AD (n=175), MCI (n=356), HC (n = 199)	Metabolomics	Sphingomyelins, PCs, acylcarnitines, amines.	serum	Sphingomyelins and PCs were altered in preclinical AD stages. Acylcarnitines and amines are altered in symptomatic stages.
[40]	developed dementia (n= 93), dementia-free (n= 1974)	Metabolomics	Amines, organic acids, lipids, and related metabolites.	plasma	Anthranilic acid ↑, glutamic acid ↑, taurine ↓, and hypoxanthine ↓ were associated with greater risk of dementia.
[41]	AD (n= 37), HC (n= 46), Normal Pressure Hydrocephalus (NPH (n= 27)), brain tumors (BT (n= 20)).	Metabolomics	The profiles of 21 metabolites were examined.	serum	No differences in 2,4-dihydroxybutanoic acid were found across AD, NPH and BT samples. Serine was increased in NPH as compared to BT. Glutamic acid ↑ in AD as compared to the HC group. In the AD group, 3-hydroxybutyrate ↓ with respect to all other groups (mean values - 30% or more), but the differences were not statistically significant.

(Table 2) contd....

Refs.	Participants	Omics Technique	Biomarker	Sample	Results
[42]	AD (n=27) MCI (n=31), HC (n=32),	Lipidomics	PC aeC32:2, PC aeC34:1, PCaaC36:5, lysoPC aC18:1, lysoPC aC16:0, one sphingomyelin.	soluble lysates of platelets	Prediction model with a diagnosis accuracy for AD versus HC and MCI of 85%.
[43]	AD, MCI and HC (n= data not found)	Metabolomics	Progesterone, lysoPCs, tryptophan, L-phenylalanine, dihydrosphingosine, phytosphingosine.	plasma	Early disease mechanisms. Tryptophan ↓, lysophosphatidylcholines ↓, dihydrosphingosine ↓ in AD.
[44]	dementia (n=143), MCI (n=145), HC (n=153)	Metabolomics	Sphingomyelins, phospholipids	plasma	Some phospholipids and metabolites were altered in MCI and dementia, PCs ↓ in AD.
[45]	AD (n=10), MCI (n=10), HC (n=15) Validation AD (n=42), MCI (n=50), HC (n=49)	Metabolomics	Fatty acids, choline and phosphatidylcholines (PCs).	plasma	3 PCs concentrations ↓ significantly in AD cases. ROC analysis of the PCs, and ApoE, produced an AUC of 0.828.
[46]	AD (n = 16), MCI (n = 7), HC (n = 84).	Lipidomics	Phosphatidylcholines (PCs).	plasma	Lower plasma concentrations of PC16:0/20:5, PC16:0/22:6, and PC18:0/22:6 are associated with poorer memory performance and decreases in brain function during aging. Dysregulation of peripheral PC metabolism may be a common feature of both AD and age-associated differences in cognition.
[47]	Dementia (n = 18), MCI (n = 77), HC (n = 126)	Metabolomics	Phospholipids/metabolites (PCs, lysoPCs, propionylcarnitine).	plasma	Some phospholipids/metabolites ↑ in AD achieving an AUC of 0.609 in predicting MCI or dementia and 0.607 in distinguishing HC from MCI or dementia.
[48]	AD (n = 30), MCI (n=14), HC (n = 30)	Lipidomics	Fatty acids (arachidic, cerotic, vaccenic, erucic, linoleic).	plasma	C20:0 arachidic ↑, C26:0 cerotic ↓, C18:1 (n-7) vaccenic ↑, C22:1 (n-9) erucic ↑, C18:2 (n-6) linoleic ↓ and mead acid ↑ showing differences between groups.
[49]	AD (n=148), HC (n = 152)	Lipidomics	Cholesteryl esters/triglycerides, phosphatidylcholines.	plasma	Model with 24 molecules classified AD patients with >70% accuracy. Lipid signatures predicted disease progression ($R^2 = 0.10$) and brain atrophy ($R^2 \geq 0.14$).
[50]	AD (n = 35), MCI (n=48), HC (n = 40),	Lipidomics	Cholesteryl esters.	plasma	A novel test (↓ cholesteryl esters in AD) set with 79.2% accuracy (81.8% sensitivity, 76.9% specificity and an AUC of 0.792).
[51]	AD (n = 35), MCI (n=38), HC (n=34)	Lipidomics	Acylcarnitines (ACCs).	plasma	Significant ↓ levels of medium-chain ACCs in AD.
[52]	High-likelihood LBD (n = 12), intermediate-likelihood LBD (n = 14), Dementia-AD (n = 18), HC (n = 21)	Lipidomics	Sphingolipid, Ceramides, Mono-hexosylceramides.	plasma	Plasma fatty acid levels did not differ by group. ↑ ceramides levels in AD.
[53]	AD (n = 43), MCI (n = 33), HC (n = 35)	Metabolomics	Acylcarnitines, amino acids, biogenic amines, sphingolipids, glycerophospholipids, sum of hexoses.	plasma	Lipid metabolites can differentiate HC from MCI and AD with relevant significance. The ratio PCs/lysoPCS ↓ in AD.
[54]	HC (n=51), MCI-1 (n=64), MCI-2 (n=13), LOAD-1 (n=57), LOAD-2 (n=33)	Lipidomics	Diacylglycerols (DAGs), Ethanolamine plasmalogens (PlsEs)	serum	Some DAGs ↑ in MCI and LOAD. Some PlsEs ↓ in both MCI and both LOAD cohorts.
[55]	AD (n=9), MCI (n=8), HC (n=12)	Metabolomics	Galactose, imidazole and acetone, creatine, 5-aminopentanoate, propionate, acetone.	saliva	Significant concentration changes in these metabolites (galactose ↓, while imidazole, 5-aminopentanoate, propionate, and acetone ↑ in AD). Logistic regression modelling allowed a statistically significant prediction of MCI and AD from HC, and MCI from AD.

(Table 2) contd....

Refs.	Participants	Omics Technique	Biomarker	Sample	Results
[26]	AD (n = 172), HC (n = 109)	Epigenomics	microRNAs: hsa-miR-9-5p, hsa-miR-29a-3p, hsa-miR-106a-5p, hsa-miR-106b-5p, hsa-miR-107, hsa-miR-125a-3p, and hsa-miR-125b-5p.	blood	Expression of hsa-miR-9-5p ↓, hsa-miR-106a-5p ↓, hsa-miR-106b-5p ↓, and hsa-miR-107 ↓ in AD. Notably, hsa-miR-106a-5p displayed, as a predictor variable, 93% specificity and 68% sensitivity.
[66]	AD (n=103), MCI (n=20), MS (n=90), HC (n=77)	Epigenomics	microRNAs.	blood	Expression of hsa-miR-151a-3p ↑, and hsa-miR-17-3p ↓ in AD. 146 deregulated microRNAs at a significance level of 0.05. With 68 microRNAs, the ROC-AUC of 0.93; 95% CI 0.89–0.96.
[67]	AD (n= 48), HC (n= 22)	Epigenomics	microRNAs: brain-miR-112, brain-miR-161, hsa-let-7d-3p, hsa-miR-5010-3p, hsa-miR-26a-5p, hsa-miR-1285-5p, hsa-miR-151a-3p, hsa-miR-103a-3p, hsa-miR-107, hsa-miR-532-5p, hsa-miR-26b-5p, and hsa-let-7f-5p.	blood	12-microRNA signature, difference between AD and HC with an accuracy of 93%, a specificity of 95% and a sensitivity of 92% (hsa-miR-107 ↓, hsa-miR-5010-3p ↑...).
[68]	Deep sequencing: AD (n=23), MCI (n=3), HC (n=23) Validation: AD (n=16) MCI (n=8), HC (n=36)	Epigenomics	Exosomal miRNA biomarkers (hsa-miR-361-5p, hsa-miR-30e-5p, hsa-miR-93-5p, hsa-miR-15a-5p, hsa-miR-143-3p, hsa-miR-335-5p, hsa-miR-106b-5p, hsa-miR-101-3p, hsa-miR-424-5p, hsa-miR-106a-5p, hsa-miR-18b-5p, hsa-miR-3065-5p, hsa-miR-20a-5p, hsa-miR-3065-5p and hsa-miR-582-5p, hsa-miR-1306-5p, hsa-miR-342-3p and hsa-miR-15b-3p).	serum	An AD-specific 16-microRNA signature was selected and adding established risk factors including age, sex and apolipoprotein ε4 (ApoE ε4) allele status to the panel of deregulated miRNA resulted in a sensitivity and specificity of 87% and 77%, respectively, for predicting AD (hsa-miR-20a-5p ↑, hsa-miR-18b-5p ↑, hsa-miR-424-5p ↑, hsa-miR-1306-5p ↓, hsa-miR-342-3p ↓, hsa-miR-15b-3p ↓ ...).
[69]	AD (n=48), HC (n=22)	Epigenomics	microRNAs: miR-26b-3p, miR28-3p, miR-30c-5p, miR-30d-5p, miR-148b-5p, miR-151a-3p, miR-186-5p, miR-425-5p, miR-550a-5p, miR-1468, miR-4781-3p, miR-5001-3p, and miR-6513-3p and downregulation in AD of let-7a-5p, let-7e-5p, let-7f-5p, let-7g-5p, miR-15a-5p, miR-17-3p, miR-29b-3p, miR-98-5p, miR-144-5p, miR-148a-3p, miR-502-3p, miR-660-5p, miR-1294, and miR-3200-3p.	blood	27 microRNAs expressed differentially between both groups (hsa-miR-26b-3p ↑, hsa-miR-28-3p ↑, hsa-miR-30c-5p ↑, hsa-miR-3d-5p ↑, hsa-miR-148-5p ↑, let-7a-5p ↓, let-7e-5p ↓, let-7f-5p ↓, let-7g-5p ↓, miR-15a-5p ↓, miR-17-3p ↓, miR-29b-3p ↓, miR-98-5p ↓, miR-144-5p ↓, miR-148a-3p ↓, miR-502-3p ↓, miR-660-5p ↓, miR-1294 ↓, and miR-3200-3p ↓ in AD).
[70]	AD (n= 107), MCI (n= 101), PDD (n= 30), VaD (n= 20)	Epigenomics	Exosomal microRNAs.	serum	Expression of exosome microR-135a and microR-384 ↑ in AD, while miR-193b ↓ in AD patients compared with HC. Exosome microR-384 was the best to discriminate AD, VaD, and PDD. ROC curve showed that the combination of miR-135a, -193b, and -384 improved the early AD diagnosis.
[30]	AD (n= 109), MCI (n= 380), HC (n= 58)	Proteomics	A1M, ApoE, BNP, and IL16, SGOT.	plasma	A set of 5 plasma proteins was differentiated between the HC group and the AD dementia group with a sensitivity of 89.36% and a specificity of 79.17%. ROC AUC of 0.92 (IL16 ↑, APOE ↓, BNP ↑, A1M ↓, SGOT ↑).
[74]	AD (n= 82), MCI (n=81), HC (n= 91)	Proteomics	Mitogen-activated protein kinase (MAPK): MAP2K4.	plasma	MAPK was positively associated with the 10-year change in CANTAB-PAL in both the individual and twin difference context. The plasma level of protein MAP2K4 was found to suggestively associate negatively (Q<0.1) with the volume of the left entorhinal cortex (MAP2K4 ↑ in AD).
[75]	AD (n=15), HC (n=15)	Proteomics	Analysis of 553 proteins.	Serum	25 differentially expressed proteins. Most of them are related with inflammatory reaction, complement and coagulation cascades, hemostasis, immune response, lipid metabolism, oxidative stress. (SERPINA3 ↓, APOA1 ↓, apolipoprotein B-100 (APOB) ↓, AZGP1 ↓, complement factor B (CFB) ↓, CP ↓, ITIH1 ↓, inter-alpha-trypsin inhibitor heavy chain H2 (ITIH2) ↓, HP ↓, SERPINA1 ↓, Gelsolin ↑ in AD).

(Table 2) contd....

Refs.	Participants	Omics Technique	Biomarker	Sample	Results
[76]	AD (n=29), MCI (n=30), HC (n=30)	Proteomics	selenoproteins.	serum	Abundant Se was due, in part, to selenoprotein P. APOE $\epsilon 4/\epsilon 4$ genotype presented higher Se concentration in serum.
[77]	AD (n = 30), DLB (n= 30), HC (n= 28)	Proteomics	Four peptides (2898, 4052, 4090, and 5002 m/z).	serum	A combination of peptides showed sensitivity of 95.0% and specificity of 93.3% for discriminating the DLB group from the AD group.
[78]	AD (n=24), MCI (n=441), HC (n=564)	Proteomics	Complement regulators C1 inhibitor and factor H, fibronectin, ceruloplasmin, and vitamin D-binding protein, apolipoproteins (AIV, B-100, H).	plasma	Levels of C1 inhibitor and factor H, fibronectin, ceruloplasmin, and vitamin D-binding protein were significantly $\downarrow$ in MCI from both cohorts. Several apolipoproteins (AIV, B-100, H) were also significantly $\downarrow$ in MCI.
[79]	AD (n=9), HC (n=10)	Proteomic	Apolipoprotein A-1, alpha-2-HS-glycoprotein, afamin, apolipoprotein A-4, fibrinogen gamma chain.	plasma	Apolipoprotein A-1 $\downarrow$, alpha-2-HS-glycoprotein $\downarrow$, and afamin $\downarrow$ in AD. Apolipoprotein A-4 $\uparrow$ and fibrinogen gamma chain $\uparrow$ in mild AD.
[80]	AD (n=33), MCI (n=24), HC (n=23) Validation: AD (n=43), MCI (n=27), HC (n=55)	Proteomics	Vascular-related proteins (alpha2-macroglobulin, apolipoprotein-A1, plasminogen activator inhibitor, RAGE, Tissue Inhibitors of Metalloproteinases-1 and Trombospondin-2, serum amyloid A, N-terminal pro-brain natriuretic peptide (NT-proBNP)).	plasma	alpha2-macroglobulin, apolipoprotein-A1, plasminogen activator inhibitor, RAGE, Tissue Inhibitors of Metalloproteinases-1 and Trombospondin-2 $\uparrow$ in AD, and serum amyloid A $\uparrow$ in AD but with a very high variance. NT-proBNP 2 $\uparrow$ in MCI and AD. NT-proBNP seems to be a useful and stable marker for diagnosing AD.
[81]	AD (n=331), MCI (n=149), HC (n=211),	Proteomics	PSA complexed to $\alpha 1$ -antichymotrypsin (PSA-act), pancreatic prohormone, clusterin, and fetuin B.	plasma	PSA-act $\uparrow$, pancreatic prohormone $\uparrow$, clusterin $\uparrow$, fetuin B $\downarrow$ in AD. Multivariate analysis found that a subset of 13 proteins predicted AD with an accuracy of AUC of 0.70.
[82]	AD (n=31), stable MCI (sMCI, n=58), Progressive MCI (pMCI, n=34), HC (n=23),	Proteomics	IgG-Fc (immunoglobulin receptors) Glycans.	plasma	Significant differences were found, complex galactosylated and sialylated forms $\downarrow$ in AD. Principal component analysis (PCA) confirmed the gender similarities and differences, and a close correlation between pro-inflammatory protein markers, AD, female pMCI, and truncated IgG-Fc glycans.
[83]	aMCI (n=73), HC (n=211)	Proteomics	TNF α , IL10, and TARC.	serum	TNF α $\uparrow$, IL10 $\uparrow$, and TARC $\uparrow$ in aMCI. The diagnostic accuracy of the biomarkers in detecting MCI was 96% (sensitivity=0.82; specificity=0.97).
[84]	AD (n = 28), MCI (n =30), HC (n= 77)	Proteomics	IL-18 and T-lymphocyte-secreted protein I-309.	serum	IL-18 $\downarrow$ and T-lymphocyte-secreted protein I-309 $\uparrow$ in AD. AD and MCI profiles had substantial overlap among the top markers, suggesting common functions in AD and MCI but differences in their relative importance.
[85]	AD (N=150), HC (n=150)	Proteomics	FABP, beta 2 microglobulin ($\beta 2M$), PPY (pancreatic polypeptide), sTNFR1, CRP, VCAM-1, thrombopoietin, $\alpha 2$ macroglobulin, eotaxin3, TNF- α , tenascin C (TNC), IL-5, IL-6, IL-7, IL-10, IL-18, I309, FVII, TARC, amyloid A (SAA), and intercellular cell-adhesion molecule-1.	Plasma and serum	The overall accuracy of the algorithm using our specific profile was superior when using serum (AUC = 0.96) versus plasma (AUC = 0.76). The 10 most important proteins in the serum proteomic profile are: IL7 $\uparrow$, TNF-alpha $\uparrow$, IL5, IL6 $\uparrow$, CRP $\downarrow$, IL10, TNC, sICAM1, FVII, I309 $\uparrow$ in AD.
[86]	AD (n=109), MCI (n=360), HC (n=58)	Proteomics	A1Micro, HBELGF, IgM, MIP-1a, PAPPa, ANG- 2, ApoA VI, C3, FasL, ILGFBP, PYY, SGOT, TTR.	plasma	A1Micro $\uparrow$, HBELGF $\uparrow$, IgM $\downarrow$, MIP-1a $\uparrow$, PAPPa, ANG- 2, ApoA VI $\uparrow$, C3, FasL $\uparrow$, ILGFBP $\uparrow$, PYY $\uparrow$, SGOT, TTR $\downarrow$ in AD. 4 different proteomic signatures, each using 5 to 14 analytes, differentiate AD from control patients with sensitivity and specificity ranging from 74% to 85%.
[87]	MCI-AD (n=139), MCI (n=120)	Proteomics	Proteins involved in cell-signalling and/or associated with a variety of disease processes, (AD, metabolic disorders, inflammation, cancer, cardiovascular disease).	plasma	The best performing model incorporated a combination of cognitive/functional markers and morphometric MRI measures and predicted progression with 80% accuracy (83% sensitivity, 76% specificity, AUC = 0.87).

(Table 2) contd....

Refs.	Participants	Omics Technique	Biomarker	Sample	Results
[88]	Stable-MCI (sMCI, n=76), Progressive MCI (pMCI, n=43)	Proteomics	alpha-2-macroglobulin (A2M), CFAB, CFAI, A1AG1, ceruloplasmin, CFAH, CFAH, fibronectin, FIBG, FIBB, FIBA, gelsolin.	plasma	A2M ↑, CFAB ↓, CFAI ↓, A1AG1, ceruloplasmin, CFAH, CFAH, fibronectin ↑, FIBG ↑, FIBB ↑, FIBA ↑, C1 inhibitor ↑, gelsolin in pMCI. The best model demonstrated the accuracy of 79% in predicting progressive MCI. A2M strongly correlates with female AD progression but not with males.
[89]	MCI (n=300)	Proteomics	Proteins associated with structural brain changes (BMP6, Eselectin, MMP10, NrCAM).	Plasma	Four proteins whose change over 1 year were associated with brain structural changes could discriminate AD from HC with sensitivity of 93%, and specificity of 92%. This model predicted MCI conversion to AD in 2 years with 94% accuracy.
[90]	AD (n=106), HC (n=51)	Proteomics	A1Micro, A2Macro, AAT, ApoE, complement C3 and PPP.	plasma	Discriminate AD from HC with a sensitivity of 85.4% and specificity of 78.6%.
[91]	AD (n=77), MCI (n=70), HC (n=50) Validation: AD (n=10), MCI (n=30), HC (n=10)	Proteomics	Protein S100A9, calgranulin B, SLAM family member 5- signalling lymphocytic activation molecule 5, leukocyte differentiation antigen CD84, CD226 antigen, allograft inflammatory factor 1, endothelial cell-selective adhesion molecule.	plasma	Protein S100A9 ↑, calgranulin B ↑, SLAM family member 5 ↓, antigen CD84 ↓, CD226 ↓, allograft inflammatory factor 1 ↓, endothelial cell-selective adhesion molecule ↓ in AD. 5-protein classifier is a promising blood test to contribute diagnosis of AD (90.1% sensitivity, 84.2% specificity, 87.9% accuracy, and AUC of 0.94).
[92]	AD (n=300), MCI (n=307), HC (n=722)	Proteomics	FABP, beta 2 microglobulin (B2M), pancreatic polypeptide, macrophage inflammatory protein 1a (MIP1a), c-reactive protein (CRP), soluble vascular cell-adhesion molecule1 (sVCAM-1), thrombopoietin, a2 macroglobulin, eotaxin3, tumor necrosis factor-alpha (TNF-a), tenascin C, interleukin-5 (IL-5), IL-6, IL-7, IL-10, IL-18, I309, FactorVII, thymus and activation-regulated chemokine, serum amyloid A, and soluble intercellular cell-adhesion molecule-1.	Serum	A2M, B2M, CRP ↑, Eotaxin ↑, FABP, FVII, I309, IL10 ↓, IL18, IL5, IL6 ↑, IL7, MIP1alpha ↓, PPY, SAA, sICAM1, sVCAM1, TARC ↑, thrombopoietin, TNC, TNF2alpha ↑ in AD. Detecting AD, PPV was 0.81, and NPV was 0.95. Detecting MCI, PPV and NPV were 0.74 and 0.93, respectively.
[93]	AD (n=30), MCI (n=30), HC (n=60)	Proteomics	Platelet proteins (tropomyosin-1, monoamine oxidase-B).	platelets	Tropomyosin-1 ↑ in female AD, monoamine oxidase-B ↑ in AD Tropomyosin-1 constitutes a gender-related and stage-dependent protein in MCI. Monoamine oxidase-B, shows a gender-independent but stage-related increase since it is unaltered in MCI subjects.
[94]	AD (n=47), svPPA (semantic-variant primary progressive aphasia) (n=92)	Proteomics	Circulatory signalling proteins (IL31, MSTN (GDF8), FLT4, KDR, FURIN, INHBA).	plasma	IL31 ↓, MSTN (GDF8) ↓, FLT4 ↑, KDR ↑, FURIN ↓, INHBA ↓ in AD. Growth-Differentiation Factor (GDF) signalling as a novel AD relevant pathway.
[95]	AD (n=3), MCI (n=3), HC (n=3),	Proteomics	Protein convertase subtilisin/kexin type 9 (PCSK9), coagulation factor XIII, A1 polypeptide (F13A1), and dermcidin (DCD).	serum	PCSK9, coagulation factor XIII, F13A1, and DCD ↑ in AD. Biomarker candidates can serve as a potential non-invasive early diagnosis.
[96]	AD (n=5), MCI (n=5), HC (n=5)	Proteomics	Mitochondrial proteins (thioredoxin-dependent peroxide reductase, myosin light polypeptide 6, and ATP synthase subunit β).	blood	Thioredoxin-dependent peroxide reductase ↓, myosin light polypeptide 6 ↑, and ATP synthase subunit β ↑ in MCI and AD. Key proteins were differentially expressed in mitochondria from AD, MCI and HC.
[97]	AD (n=108), MCI (n=360), HC (n=53) Validation: AD (n=6), HC (n=16), Cognitive complaint (CC, n=18), Early MCI (EMCI, n=10), Late MCI (LMCI, n=9)	Proteomics	H-related protein 1 (CFHR1), interleukin-6 receptor, chemokine CC-4, angiotensin-converting enzyme, and angiotensinogen.	Plasma	Genetic variation takes on new importance and they are considered in interpretation of proteomic results. CFHR1 level accounting for 40 percent of total variation of the protein level. An association between this protein and some SNPs (rs6677604, rs6677604, rs7517126) were found. 78 other SNP-protein associations in the ADNI sample exceeded genome-wide significance. These results confirmed previously identified gene-protein associations for interleukin-6 receptor, chemokine CC-4, angiotensin-converting enzyme, and angiotensinogen.

Abbreviation: FABP: Fatty acid-binding protein. LBD: Lewy Body Dementia. miR: microRNA. PDD: Parkinson's disease with dementia. sTNFR1: soluble tumor necrosis factor receptor 1. TARC: Thymus and activation-regulated chemokine. VaD: Vascular Dementia.

and reliable AD biomarkers, and to study the progression of the disease, but further research looking for specific AD biomarkers among neurodegenerative diseases is required.

Our group has validated analytical methods based on chromatography coupled with mass spectrometry (UPLC-MS/MS) for the determination of oxidative stress biomarkers (isoprostanes, isofurans from arachidonic acid, neuroprostanes, neurofurans from docosahexaenoic acid, dihomoisoprostanes, dihomoisofurans from adrenic acid) in different biofluids (serum, plasma, whole blood, urine, CSF) [56-58]. These biomarkers are being studied in patients with MCI-AD and mild dementia-AD, as well as in healthy participants of the same ages [59, 60]. By now, the results obtained are promising, especially for neuroprostanes and dihomoisoprostanes, which reflect the oxidation of highly concentrated lipid components in the grey and white brain matter. These biomarkers have potential interest in diagnostic and prognostic issues, and biomarkers related to molecular mechanisms could be therapeutic targets in further pharmacological developments.

3.2. MicroRNAs

Among epigenetic biomarkers, the microRNAs constitute a key element in cell signalling pathways. In recent years, they have been postulated as powerful biomarkers for the diagnosis of neurodegenerative diseases [61]. In fact, there is evidence that they could be more sensitive than messenger RNA, or even proteins used as clinical markers [62].

Recent epigenomic studies in AD using NGS have shown the existence of microRNAs with altered expression in both brain and CSF [63, 64]. However, other studies carried out in peripheral biofluids using quantitative Reverse transcription Polymerase chain reaction (qRT-PCR) provided inconsistent results [65]. Some studies summarized in Table 2 have identified statistically significant differences in the regulation of microRNAs in AD patients compared with HC subjects [66-69], as well as in other neurological diseases [67, 70]. Nevertheless, some discrepancies regarding microRNAs levels and expression patterns may be explained by confounding factors, such as the different ethnic groups, sample handling, and bias or random findings because small samples have been employed in each study. In fact, it seems that medication and disease duration can influence on the microRNAs' profiles [26, 66]. In this sense, a previous study found a limitation in the ability to diagnose patients with late AD from the microRNAs identified [68]. In addition, some exosomal microRNAs (miR-135a and miR-384) were up-regulated in AD patients compared with HC subjects, while another (miR-193b) was down-regulated in AD, but these microRNAs were not specific of AD, and can be also found altered in Parkinson's disease with dementia and vascular dementia [70]. According to this, epigenomics constitutes a potentially useful tool in the development of microRNA signatures and cost-effective diagnostic models with satisfactory accuracy (89-96 %) [66-68]. However, no study evaluating the diagnostic performance of different microRNAs expression between MCI-AD and HC participants was found. Therefore, further studies in blood or exosome samples from well-defined MCI-AD and HC subjects, and following a standard

protocol, are required to achieve consensus on microRNAs as specific biomarkers in AD. On the other hand, lncRNAs can also regulate gene expression at epigenetic level, but few studies have focused on the role of lncRNAs as minimally invasive AD biomarkers [71, 72].

3.3. Proteins

Proteins involved mainly in synapse and immunity functions are important AD biomarkers. In a recent study in CSF samples from MCI-AD patients high levels of several proteins (Chromogranin A, neurexin3, neuropentraxin1) were found, reflecting early events in the physiopathology of AD [73]. Another study found significant relationships between AD and high levels of certain proteins in blood (kinases, pancreatic polypeptide, β -microglobulin (2), carcinoembryonic antigen, superoxide dismutase) [74], but its replication failed probably due to the technical differences with other studies, or the small sample sizes that provide insufficient statistical power.

Recent proteomic studies (Table 2) have identified proteins as potential early AD biomarkers in plasma or serum samples. Most of them are related to inflammatory reaction, complement and coagulation cascades, hemostasis, immune response, lipid metabolism, and oxidative stress [75]. Actually, selenoprotein P was found at higher level in MCI than in HC [76], and some apolipoproteins were downregulated in MCI (apolipoprotein E, apolipoprotein A-1, alpha-2-HS-glycoprotein, afamin, AIV, B-100, H) [26, 77-79], while another (apolipoprotein A-4) was upregulated in MCI [79]. Although most of these proteins were reported as potential AD biomarkers in some studies [78, 79], Muenchhoff *et al.* found a significant decrease of fibronectin and C1 inhibitor at the MCI stage that would require further validation [78]. Also, a combination of peptides allowed the development of a satisfactory diagnostic model discriminating between dementia with Lewy bodies (DLB) and AD groups [77], and N-terminal pro-brain natriuretic peptide (NT-proBNP) would be a useful MCI and AD biomarker, as it was confirmed in an external validation [80]. Similarly, multivariate analysis confirmed correlations between some proteins (PSA, clusterin, fetuin B and glycans) and AD [81, 82]. As regards to inflammatory pathways, some related proteins (TNF α , IL5, IL1, IL7, L10, IL18, TARC) were found deregulated in MCI-AD patients [83-85], these MCI profiles were different from AD profiles [83, 84], corroborating peripheral inflammation as an important factor in the disease progression. Most of the AD diagnostic models developed in literature from omic tools were based on proteomic signatures and showed satisfactory accuracy (70-94%) [30, 38, 81, 86-89], as well as high sensitivity and specificity (74-85%) [86, 90, 91]. Regarding discrimination between MCI patients and controls, a diagnostic model developed from TNF α , IL10, and TARC biomarkers showed satisfactory indexes (accuracy 96%, sensitivity 82%; specificity 97%) [83]; NT-proBNP protein yielded high accuracy (AUC-ROC 0.777) and sensitivity (79.2%), whereas the specificity was quite lower (64%) [80]. Another study that evaluated the blood screen obtained a positive predictive value and a negative predictive value of 0.74 and 0.93, respectively [92]. A biomarker profile, based on IL-18 and T-lymphocyte-secreted protein I-309, showed

poor accuracy for the MCI group (AUC-ROC 0.58), while it was highly accurate for the AD group (AUC-ROC 0.94) [84]. On the other hand, there is evidence that human blood proteome has gender specific profiles [82], as it was observed for A2M that strongly correlates with female AD progression but not with males [88], and for tropomyosin-1 in platelets [93].

In general, the proteomics findings were characterized by high AD specificity and they contributed importantly in the advance of knowledge about the physiopathological mechanisms involved. For instance, a combination of two peptides (1737 and 5002 m/z) showed sensitivity of 95.0% and specificity of 93.3% for discriminating the dementia with Lewy bodies group from the AD group [69], as well as some polypeptides with 79% of accuracy differentiating between stable MCI and progressive MCI [88]. However, different proteomics signatures were obtained from these results, indicating inconsistency and low reproducibility among studies. As regards sources of variability, it is important to highlight ethnicity [84, 85], clinical heterogeneity in participants cohorts [77, 82, 88, 94], poorly specific AD diagnosis based on neuropsychological testing and not checked by means of specific CSF or PET biomarkers [88, 91, 92], different stages of AD [94-96], small sample sizes [75, 76], and different blood fractions (plasma, serum) [85]. Moreover, gene polymorphism constitutes an important characteristic that could contribute to different levels of some proteins in different reports [75]. Other important limitations in omic technologies are the use of non-standardized experimental protocols, and the high variability of assays platforms and analytical techniques [29, 30, 85, 88, 91]. Moreover, there are few studies validating the potential biomarkers in independent samples [80, 81, 91, 95, 97]. Therefore, more studies are needed to validate changes of some proteins in AD, and develop reliable biomarkers with clinical utility.

3.4. Novel Therapeutic Targets in Early AD Treatment

In the initial phases of AD, differentiation between patients and the general population, which presents a great variability, harbours great difficulty. However, an early and accurate diagnosis would be required, since it is precisely in the early stages of AD when available treatments show higher efficacy.

The previously described omics-based biomarkers constitute an important source of information to develop novel and effective diagnostic tools and therapeutic strategies. From the metabolomics pathways with relevancy in AD development, lipid metabolism impairment plays an important role. In fact, lipid signatures have been developed in order to predict the disease progression [49]. Since some lipid metabolites such as acylcarnitines, phosphatidylcholines, plasmalogens, sphingolipids, triglycerides, showed lower levels in plasma from AD patients than in HC subjects [23, 37, 46, 51, 54], they could play a significant role in the modulation of brain metabolism. Therefore, a potential therapeutic strategy could be based on special diets and/or pharmacological preparations aimed at maintaining the normal levels of these metabolites from early AD stages. In addition, some studies have shown that amino acids and neurotransmitters mecha-

nisms were impaired in AD. In fact, some of them showed higher levels of metabolites, such as, taurine, glutamic acid, alanine, methionine, and asparagine [38, 40, 41], while in other works lower levels of glutamic acid and aspartic acid, were found in AD [38]. In the same way, some amines (2-amino adipic acid, tyrosine) were under-expressed in AD [37]. In some cases, discrepancy can be due to differences in methodology, as well as the lack of validated analytical methods.

Taking into account the results obtained in the epigenomic studies, there are some microRNAs that show different levels between AD and HC groups, so modifications in the expression of genes regulated by these microRNAs could be the base for a potential therapeutic strategy, trying to maintain the expression of these genes at levels close to HC subjects.

From the proteomic studies, some downregulated proteins in MCI-AD were identified (apolipoprotein A-1, AIV, B-100, H, alpha-2-HS-glycoprotein, and afamin, complex galactosylated and sialylated, C1 inhibitor and factor H, fibronectin, ceruloplasmin, and vitamin D-binding protein) [78, 79, 82]. These findings may indicate that these proteins, involved in some pathways, such as, inflammation, coagulation, hemostasis, immune response, lipid metabolism, and oxidative stress could act as therapeutic targets for AD treatment.

The main limitation of new omics-based biomarkers is their still limited clinical use. In fact, few potential biomarkers have been clinically validated may be due to experimental difficulties (*e.g.* there is not a validation protocol), and high economic cost (*e.g.* large scale and an in-depth investigation of critical parameters). Also, once a test has been validated in the laboratory, it is necessary to determine its validity in the real clinical practice. Another important limitation in the development of omics-based biomarkers is the influence of diverse aging brain comorbidities coexisting in AD, and they can be differentiated into non-degenerative processes (*e.g.* general somatic, metabolic or vascular secondary brain impairment), or degenerative brain diseases (*e.g.* Parkinson disease, frontal lobe type degenerations). However, studies reviewed in this work did not evaluate the impact of potential AD co-morbidities. On the other hand, differentiation between distinct degenerative processes can be especially difficult in the absence of specific diagnosis biomarkers, so further research on omics-based biomarkers using well defined groups could contribute significantly in this field. Finally, regarding the high heterogeneity of AD, the integration of omics data obtained using different technologies constitutes a promising approach to advance in the knowledge of the AD physiopathology, and subsequently in the development of an efficient therapeutic strategy. Nevertheless, there is a lack of integration studies, so further research in this line is required.

CONCLUSION

Currently, most of the research lines are focused on the early, accurate and safe diagnosis of Alzheimer disease, and in the development of efficient therapeutic strategies. Omics techniques can allow identifying biomarkers for early diag-

nosis or prognosis, and they can aid in the development of new therapeutic strategies. Nowadays, there is little translational experience in the application of the results of omic techniques to the clinical practice. In fact, no comparative studies between new omics-based biomarkers and standard diagnosis biomarkers (CSF amyloid, tau, p-tau, atrophy or hypometabolism on neuroimaging procedures) have been found in the literature, and few potential biomarkers have been clinically validated. As an improvement, research using strictly selected case and control groups, an appropriate sample size, as well as standardized and repeatable laboratory techniques, could provide reliable information on the molecular mechanisms that occur in the affected tissue, related tissues and at a systemic level. On the other hand, some omics variations could be specific of AD, while others could constitute unspecific markers of neurodegeneration or neurological damage of any kind. In this sense, studies including other neurodegenerative diseases and diverse conditions causing neurologic damage will be necessary to elucidate these possibilities.

To conclude, omics biomarkers can provide an easy, non-expensive and non-invasive approach useful in clinical practice replacing actual expensive neuroimaging techniques, time consuming neuropsychological assessment or invasive sampling procedures. In addition, omics based-biomarkers are not limited to diagnostic or prognostic issues, but also they can be used as risk biomarkers, group definition biomarkers, as well as definite or surrogate therapeutic biomarkers. Finally, there is an important imbalance between the scientific production and the lack of clinically validated biomarkers. Therefore, a number of improvements are required in further works developing reliable and cost-effective diagnosis, as well as effective therapies: a multi-omic approach to advance in the knowledge of the complex AD physiopathology, the identification of early and specific AD biomarkers in non-invasive biofluids using well-defined cohorts, the development and validation of low-cost analytical methods, and the clinical validation of potential biomarkers.

LIST OF ABBREVIATIONS

A1M	=	A-1-microglobulin	CRP	=	C-reactive protein
AAT	=	Alpha-1 Antitrypsin	CXCL	=	Chemokine that contains a C-X-C motif
ACC	=	Acylcarnitine	DAG	=	Diacylglycerol
AD	=	Alzheimer Disease	DAT	=	Dementia of Alzheimer-type
aMCI	=	Amnesic mild cognitive impairment	DLB	=	Dementia with Lewi Bodies
ANG-2	=	Angiopoietin-2.	EGF	=	Epidermal growth factor
ApoE	=	Apolipoprotein E	EMCI	=	Early mild cognitive impairment
APP	=	Amyloid beta A4 precursor protein	G-CSF	=	Granulocyte-colony stimulating factor
BACE1	=	Beta-secretase1	GDNF	=	Glial-derived neurotrophic factor
BNP	=	Brain natriuretic peptide	HC	=	Healthy control
BT	=	Brain tumours	ICAM-1	=	Intercellular adhesion molecule-1
CC	=	Cognitive complaint	ICP-MS	=	Inductively coupled plasma-mass spectrometry
CCL	=	Chemokine that contains a C-C motif	IGFBP-1	=	Insulin-like growth factor-binding protein-6
CI	=	Confidence interval	IgG	=	Immunoglobulins
			IL	=	Interleukin
			LC	=	Liquid chromatography
			LMCI	=	Late mild cognitive impairment
			LOAD	=	Late onset Alzheimer Disease
			LysoPC	=	Lyso-phosphatidylcholines
			MCI	=	Mild Cognitive Impairment
			M-CSF	=	Monocyte-colony stimulating factor
			MRI	=	Magnetic Resonance Imaging
			MS	=	Mass spectrometer
			MS	=	Multiple Sclerosis
			NC	=	Normal control
			NGS	=	Next generation sequencing
			NPH	=	Normal Pressure Hydrocephalus
			NPV	=	Negative predictive value
			NT-proBNP	=	N-terminal pro-brain natriuretic peptide
			PC	=	Phosphatidylcholine
			PCA	=	Principal component analysis
			PDD	=	Parkinson's disease with dementia
			PDGF-BB	=	Platelet-derived growth factor BB
			PLS-DA	=	Partial least squares – discriminant analysis
			pMCI	=	Progressive mild cognitive impairment
			PPP	=	Pancreatic Polypeptide
			PPV	=	Positive predictive value

PSA	=	Prostate-specific antigen
PSEN	=	Presenilin
qRT-PCR	=	Quantitative Reverse transcription Polymerase chain reaction
ROC AUC	=	Receiver operating characteristic - Area under curve
SEC	=	Size exclusion chromatography
SGOT	=	Serum glutamic oxaloacetic transaminase
sMCI	=	Stable mild cognitive impairment
SNP	=	Single nucleotide polymorphism
svPPA	=	Semantic-variant primary progressive aphasia
TARC	=	Thymus and activation-regulated chemokine
TNF α	=	Tumour necrosis factor-alpha
TRAIL-R4	=	TNF-related apoptosis-inducing ligand receptor-4
VaD	=	Vascular Dementia

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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