REVIEW

MicroRNAs as diagnostic and therapeutic tools for Alzheimer's disease: advances and limitations

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Abstract

Alzheimer's disease (AD) is the most common age-related, progressive neurodegenerative disease. It is characterized by memory loss and cognitive decline and responsible for most cases of dementia in the elderly. Late-onset or sporadic AD accounts for > 95% of cases, with age at onset > 65 years. Currently there are no drugs or other therapeutic agents available to prevent or delay the progression of AD. The cellular and molecular changes occurring in the brains of individuals with AD include accumulation of β-amyloid peptide and hyperphosphorylated tau protein, decrease of acetylcholine neurotransmitter, inflammation, and oxidative stress. Aggregation of β-amyloid peptide in extracellular plaques and the hyperphosphorylated tau protein in intracellular neurofibrillary tangles are characteristic of AD. A major challenge is identifying molecular biomarkers of the early-stage AD in patients as most studies have been performed with blood or brain tissue samples (postmortem) at late-stage AD. Subjects with mild cognitive impairment almost always have the neuropathologic features of AD with about 50% of mild cognitive impairment patients progressing to AD. They could provide important information about AD pathomechanism and potentially also highlight minimally or noninvasive, easy-to-access biomarkers. MicroRNAs are dysregulated in AD, and may facilitate the early detection of the disease and potentially the continual monitoring of disease progression and allow therapeutic interventions to be evaluated. Four recent reviews have been published of microRNAs in AD, each of which identified areas of weakness or limitations in the reported studies. Importantly, studies in the last three years have shown considerable progress in overcoming some of these limitations and identifying specific microRNAs as biomarkers for AD and mild cognitive impairment. Further large-scale human studies are warranted with less disparity in the study populations, and using an appropriate method to validate the findings.

Key Words: *Alzheimer's disease; mild cognitive impairment; microRNAs; biomarkers; blood; brain tissue; cerebrospinal fluid; humans; animal models*

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Introduction

Alzheimer's disease (AD) is the most common age-related, progressive neurodegenerative disease. It is characterized by memory loss and cognitive decline and accounts for most cases of dementia in the elderly. Currently more than 35 million people globally have AD and it is forecast to affect 1% of all people worldwide by 2050 (Brookmeyer et al., 2007; Querfurth and LaFerla, 2010; Thies and Bleiler, 2011). AD patients have been diagnosed as probable AD based on clinical examination, magnetic resonance imaging, positron emission tomography, cerebrospinal fluid (CSF) assays, and neuropsychological testing which includes cognitive performance (McKhann et al., 1984; McKhann et al., 2011). There are two major forms of AD, early-onset (familial) and late-onset (sporadic). Early-onset AD is rare, whereas late-onset AD accounts for > 95% of cases (Bali et al., 2012). Late-onset AD is defined as AD with age at onset > 65 years, while early-onset AD is AD with age at onset from 30 years to 65 years (Piaceri et al., 2013). Etiology involves a combination of genetic (70%) and environmental factors (30%) (Dorszewska et al., 2016). Many genes have been shown to be involved in the development of late-onset AD including *ABCA7*, *APOE*, *BIN1* (Barber, 2012). At present there are no drugs or other therapeutic agents available to prevent or delay the progression of AD. No biomarkers have yet been confirmed for the early detection of AD before the onset of irreversible neurological damage (Reddy et al., 2017). Multiple cellular and molecular changes occur in the brains of individuals with AD. These include neuronal and synaptic loss, mitochondrial damage, production and accumulation of β-amyloid peptide (Aβ) and hyperphosphorylated tau protein, decrease of acetylcholine neurotransmitter, inflammation, and oxidative stress. Aggregation of Aβ peptide in extracellular plaques and the hyperphosphorylated tau protein in intracellular neurofibrillary tangles (NFTs) are characteristic of AD (Serrano-Pozo et al., 2011). MicroRNAs are small noncoding RNAs (~22 nucleotides) and involved in each of the cellular changes in AD. They act by binding to the 3′-untranslated region (3′-UTR) of their target mRNAs and interfere with gene regulation and translation, and cause mRNA destabilization or degradation (Guo et al., 2010; Reddy et al., 2017). They have been detected in blood, CSF, saliva and urine, and also in blood cells such as mononuclear cells and erythrocytes.

A major challenge is identifying molecular biomarkers characteristic of the early-stage AD in patients as most studies have been performed with blood or brain tissue samples (postmortem) at late-stage AD. Towards this end, subjects with mild cognitive impairment (MCI) almost always have the neuropathologic features of AD (Morris et al., 2001; Morris and Cummings, 2005; Garcia-Ptacek et al., 2016)

and could provide important information. About 50% of MCI patients progress to AD (Sewell et al., 2010). Also the clinical-epidemiological relationship between AD and major depressive disorder (MDD) suggests they might have common neurobiological abnormalities (Rodrigues et al., 2014; Mendes-Silva et al., 2016). The pattern of microRNA regulation in each disorder could help with elucidating AD pathomechanism and also elucidate minimally or noninvasive, easy-to-access biomarkers.

The currently available biomarkers of AD are detected either by CSF analysis of Aβ and tau protein levels (Mattsson et al., 2009), brain imaging using positron emission tomography to detect Aβ deposits (Vlassenko et al., 2012), or postmortem gross specimen analysis and histology of brain sections (Braak et al., 2006). All of these are expensive, invasive, require skilled expertise to perform and interpret, or time-consuming (Lusardi et al., 2017), and only available in a small number of cases. The CSF levels of Aβ42 isoform and tau protein and particularly the ratio of tau/Aβ42 and phospho-tau/Aβ42 are useful for predicting the risk of progressing from MCI/very mild dementia to AD (Holzman, 2011; Fagan and Perrin, 2012) and have been used to identify MCI patients diagnosed with probable early AD (Najaraj et al., 2017). However, blood contamination occurs in up to 20% of CSF samples collected by lumbar puncture (Aasebø et al., 2014) and may be a confounding factor affecting Aβ42 levels (Bjerke et al., 2010). Also CSF collection by lumbar puncture may not be easily accomplished in elderly patients due to lumbar disc degeneration with narrowing of the intervertebral spaces.

Increasing evidence suggests that microRNAs are dysregulated in neurodegenerative diseases such as AD, Parkinson's disease, and stroke. Circulating microRNAs within blood may be characterized and used as minimally invasive diagnostic biomarkers. They may facilitate the early detection of the disease and potentially the continual monitoring of disease progression and allow therapeutic interventions to be evaluated. Four recent reviews have been published on studies of microRNAs in AD. They were by Batistela et al. (2017), Mendez-Silva et al. (2016), Van Giau and An (2016), Wu et al. (2016) and, where reported, included studies up to June 2015 and September 2015. Each of the reviews identified areas of weakness or limitations in the reported studies (**Table 1**) and the findings were often conflicting. The limitations included small size and heterogeneity of study populations, no postmortem confirmation of AD diagnosis or assay of other biomarkers, variations in analytical methods and platforms used and validation of findings, different methods of normalization, statistical testing and data processing, selection of cognitively normal controls (NC) and no confirmation that they did not develop clinical cognitive impairment. Also Ren et al. (2016) indicated that in many of the previous studies on microRNAs in brain, CSF, and blood from AD patients, most samples were from patients being treated with acetylcholinesterase inhibitor, which could have a confounding effect on analysis, rather than de novo drug-naïve patients. Interestingly, differential correlation analysis using paired microRNAs detected different and more sensitive plasma microRNA biomarkers for MCI than were found by single molecule analysis using a *t*-test (Kayano et al., 2016). Plasma paired microRNA sets were shown to have high sensitivity and specificity for differentiating MCI from age-matched control, and also differentiated AD from age-matched control. In addition, the paired microRNA sets detected MCI in 7 of 10 elderly patients at asymptomatic stage 0.5–5 years preceding the clinical diagnosis (Sheinerman et al., 2012).

There is range of disease states from mild dementia to se-

Table 1 Limitations of previous studies of microRNAs in Alzheimer's disease (AD) as indicated in four recent reviews

Limitation	Batistela et al. (2017)	Mendez-Silva et al. (2016)	Van Giau and An (2016)	Wu et al. (2016)
A lack of reproducibility of findings can be attributable to many variables such as the size and homogeneity of study population, including age, sex, ethnicity, and the stage of disease; selection of adequate controls; analytical methods *i.e.*, next generation sequencing (NGS), microarray or real-time quantitative polymerase chain reaction (RT-qPCR); different methods of normalization; statistical testing and data processing	√	√	√	√
There are few studies profiling microRNAs in more than one neurodegenerative disease	√	√		
MicroRNAs and results need to be validated in large independent longitidinal studies including subjects acroos the lifespsan trajectory with a comprehensive cognitive assessment to ascertain the development of AD or other neurodegenerative diseases		√	√	
Studies evaluated young and middle-aged adults limiting the generalization of findings to older adults		√		
There is no current active clinical trial on microRNA-based therapeutics in AD		√		
Whether microRNAs can be used in AD diagnosis alone or in combination with other AD biomarkers (amyloid and tau, Aβ42) warrants further investigation			√	√
The studies in the review had no postmortem confirmation of diagnosis, therefore cannot confidently ascertain if changes in microRNA expression were due to AD pathology				√
No follow-up was performed to ensure the cognitively normal controls did not develop clinical cognitive impairment				√

√ indicates that the parameter was mentioned as a limitation in the review article. Aβ: β-Amyloid peptide.

vere impairment within AD patients. The extent of cognitive decline is commonly estimated by the Mini-Mental State Examination (MMSE) and scores range from 0–30. Probable AD patients can be classified as having mild (MMSE scores 21–24), moderate (MMSE scores 10–20), or severe dementia (MMSE scores 0–9), while normal subjects have MMSE scores 25–30 (Galasko, 1998). The MMSE does not have sufficient sensitivity to detect cognitive decline at the MCI stage (MMSE mean score 27.0, reported by Nasreddine et al., 2005), and other cognitive assessment examinations have to be used. In the Montreal Cognitive Assessment (MoCA) examination, scores range from 0–30 and a score of ≥ 27 is considered normal. In a study by Nasreddine et al. (2005) normal subjects had a mean MoCA score of 27.4, MCI individuals a mean MoCA score 22.1 (range 17–23), and probable AD patients a mean MoCA score 16.2 (range 0–24). Identifying biomarkers capable of distinguishing MCI from the normal elderly and probable AD cases, as well the transition between the stages of mild, moderate, and severe cognitive impairment in AD, is an important need in indicating dysregulated signaling pathways and suggesting possible potential treatment strategies.

The goal is to identify microRNA biomarkers of high sensitivity and specificity in early-stage AD, and paired microRNA analysis or using a panel of microRNAs may be an important method to accomplish this. We have performed a PubMed literature search of articles published in the period January 2016–June 2018 on microRNAs in AD and also MCI when included as prodomal or early-stage AD. This is to provide further information on microRNA dysregulation in AD and to assess whether the weaknesses or limitations identified in previous studies have been taken into account or overcome.

Braak NFT staging of AD pathology

Histomicroscopic evaluation of AD-related pathology is based on the deposition of hyperphosphorylated tau protein within select neuronal types in specific nuclei or areas. The staging of AD-related neurofibrillary pathology using silver-stained sections 100 μm thickness has been described (Braak and Braak, 1991). A more recent description employs sections 5–15 μm thickness and immunostained for hyperphosphorylated tau protein with monoclonal antibody AT8 (Braak et al., 2006). Six stages can be recognized in the progression of AD and they are usually grouped under stages I–II, III–IV, V–VI with the major characteristics being as follows. In stage I, lesions develop in the transentorhinal region. Subcortical nuclei (viz. locus coeruleus, magnocellular nuclei of the basal forebrain) occasionally exhibit the earliest changes in the absence of cortical involvement. The transentorhinal region is the first site in the cerebral cortex to be involved. The entorhinal region proper is not or minimally involved. In stage II, lesions extend into the entorhinal region, while in stage III, lesions extend from the transentorhinal region to the neocortex of the fusiform and lingual gyri and then diminish markedy beyond this region. In stage IV, lesions progress more widely into neocortical association areas. Stage V is characterized by lesions appearing in previously uninvolved areas and extending widely into the first temporal convolution, and into high order association areas of frontal, parietal, and occipital neocortex (peristriate region). For stage VI, lesions reach the secondary and primary neocortical areas and extend into the striate region of the occipital lobe.

MicroRNAs in AD and MCI

A total of nineteen research articles were found in the PubMed search of which two had used blood, one PBMNs, four serum, four plasma, five CSF, two brain tissue, and one olfactory mucosa samples (**Table 2**). While most had used a validation method, which was usually real-time quantitative polymerase chain reaction (RT-qPCR), there were several studies where this was not performed. This is especially important as in many cases the validation findings were not in complete agrement with the screening results. Also when reported, all the studies had included both male and female subjects for AD, MCI and NC, but often there was a disparity in the proportions of the two genders in the different groups and also in their mean ages. In nearly all the studies there was no follow-up of MCI and NC individuals. Moreover, while several of the studies had involved quite large cohorts of AD and NC patients, the majority were performed with much smaller cohorts. Only a few studies had reported on ethnicity or medications being used by the patients. Inclusion and exclusion criteria were not reported in many of the studies. These are all important limitations as identified in the earlier reviews (**Table 1**) and many of these constitute confounding factors. Not all of the studies had used receiver operating characteristics (ROC) analysis with area under curve (AUC) values to establish which microRNAs are good or fair tests to distinguish AD from NC or MCI from NC patients.

The imporant findings from the research articles in the PubMed search are summarized as follows.

Blood samples

Chang et al. (2017) employed integrated analysis of microarray datasets of blood of AD and NC subjects and identified differentially expressed genes in AD that were regulated by miR-26b-5p, -103a-3p, -107, -26a-5p. However, using bloods from 3 AD and 3 NC patients, the upregulated expression of miR-26b-5p and miR-26a-5p by RT-qPCR was inconsistent with the integrated analysis results. In a study with large-sized groups, Keller et al. (2016) combined a screening/discovery set (USA) and a next-generation sequencing (NGS) validation study set (Germany, with 49 AD and 55 NC) to show upregulated expression of miR-151a-3p and downregulated expression of miR-17-3p for AD compared with NC subjects. ROC analysis indicated miR-151a-3p was a fair test to distinguish AD from NC (AUC 0.74). A marked upregulation of microRNAs was found for AD compared with MCI with miR-30c-5p having the lowest *P* value. This study had included Aβ42, t-tau and p-tau markers to aid confirmation of AD.

PBMN samples

By qPCR validation, Ren et al. (2016) showed miR-339 and miR-425 were upregulated and miR-639 was downregulated

Table 2 MicroRNAs in human patients with Alzheimer's disease (AD) or mild cognitive impairment (MCI)

Reference	No. of patients, gender, age, ethnicity/ country, inclusion/exclusion criteria	Sample for assay	Screening/discovery method	Validation method	Dysregulated miRNAs	Functional outcomes, receiver operating characteristics (ROC) analysis, specificity/sensitivity	Conclusion, comments
Blood, peripheral blood mononuclear cells (PBMNs)							
Chang et al. (2017)	AD, 3; normal controls (NC), 3, China	Blood	Integrated analysis of microarray datasets in blood of AD and NC, and data with drug stimulation or transfection excluded.	Real-time quantitative polymerase chain reaction (RT-qPCR)	Upregulated expression of miR-26b-5p, -103a-3p, -107, -26a-5p was found in AD compared with NC.	Differentially expressed genes *PLCB2*, *CDK5R1*, *LRP1*, *NDUFA4*, *DLG4* are regulated by miR-26b-5p, -103a-3p, -107, -26a-5p	The upregulated expression of miR-26b-5p and miR-26a-5p in AD compared with NC by RT-qPCR was inconsistent with the results of the integrated analysis.
Keller et al. (2016)	AD, 27 male (M)/22 female (F), 70.7 years, mean age of onset 68.7 years, MMSE 21.6, MoCA 15.9; NC, 26M//29F, 67.3, NA, MMSE 29.5, MoCA 28.8. β-amyloid (Aβ)42, t-tau and p-tau markers were consistent with AD. Medications taken by some AD included antidementive and antihypertensive drugs. Germany	Blood	Screening previously carried out using cohort of subjects. USA	Next-generation (deep-) sequencing (NGS) validation using German cohort.	68 microRNAs overlapped in the US cohort and the validation cohort. MiR-151a-3p had the lowest p value in comparing AD with NC in the discovery and validation study combined. Expression of miR-151a-3p was upregulated while that of miR-17-3p was downregulated for AD compared with NC. A substantial upregulation of miRNAs was found for AD compared with MCI (n = 20), with miR-30c-5p having the lowest P value.	ROC indicates miR-151a-3p is a fair test to distinguish AD from NC (AUC 0.74). MiR-17-3p had the largest AUC 0.77, while the 200 marker signatures had AUC 0.84. For the best single marker accuracy, specificity and sensitivity were 0.733, 0.753 and 0.718, respectively. Using signatures, the accuracy improved to 0.782, specificity and sensitivity were 0.689 and 0.876.	No significant differences in miRNAs were found between AD with and without antIdementive and antihypertensive treatment after adjustment for multiple testing. Also no significant differences in miRNAs were found for AD with disease duration < 2 years and those with disease duration > 2 years. 14 validated target genes were found of at least 5 of 33 miRNAs overlapping in both studies: *VEGFA*, *DICER1*, *AGO1*, *PTEN*, *CDKN1A*, *APP*, *RBI*, *CCND1*, *CCND2*, *WEE1*, *IL13*, *HMGA2*, *TNFRSF1OB*, *MYC*.
Ren et al. (2016)	AD, mild late onset 4, > 65 years, MMSE 20-23; AD, severe late onset, 4, MMSE < 10; NC, 4. In addition, AD, moderate 39, ≥ 60 years, MMSE 10–19, drug-naive; severe 6, MMSE < 10, drug-naïve; NC 41, ≥ 60 years. All AD were diagnosed as probable AD based on clinical and neuropsychological examinations. All AD and NC were unrelated Chinese Han. China	Peripheral blood mononuclear cells (PBMNs)	Microarray	qPCR	By qPCR, miR-339 and miR-425 were upregulated and miR-639 was downregulated in AD PBMNs compared to NC PBMNs. No significant correlations were found between the 3 microRNAs and serum Aβ40 concentration or MMSE scores	ROC indicates miR-339 is a fair test to distinguish AD PBMN from NC PBMN (AUC 0.768) and miR-425 is a good test to distinguish AD PBMN from NC PBMN (AUC 0.868), with sensitivity/specificity of 0.80 and 0.60 for miR-339, and 0.80 and 0.83 for miR-425. MiR-639 is a poor test to distinguish AD PBMN from NC PBMN (AUC 0.287)	MiR-425-5p and miR-339-5p each significantly decreased the activity of BACE1-UTR reporter, but miR-639 did not affect reporter activity. BACE1 might be a direct target of miR-425-5p and miR-339-5p.
Blood serum							
Wu et al. (2017)	AD, 32M/23F, mean age 72.1 years, MMSE mean score 12.8; NC, 30M/20F, 73.0 years, MMSE 28.9. Patients receiving heparin therapy at time of blood collection, patients diagnosed with any non-AD type of dementia, patients suffering from other diseases such as AMI, cancer, PD were excluded. China	Serum	Next- generation (deep-) sequencing (NGS)	RT-qPCR	By RT-qPCR, expression levels of miR-146a-5p, -106b-3p, -195-5p, -20b-5p, -497-5p were higher, while those of miR-125b-3p, -29c-3p, -93-5p, -19b-3p were lower in AD than in NC subjects.	Both RNA deep-sequencing and RT-qPCR indicated lower serum levels of miR-29c-3p and miR-19b-3p in AD. Computational analysis predicted that 3'-UTR of STAT 3 mRNA is a target of both miR-29c-3p and miR-19b-3p.	Serum levels of miR-29c-3p and miR-19b-3p are possible biomarkers for AD, but no ROC/ AUC analysis to indicate whether they are good tests to differentiate AD from NC.
Kumar et al. (2017)	AD, 6M/5F, 76.3 years, MMSE 22.8; MCI, 10M/10F, 74.1 years, MMSE 25.1; NC, 6M/12F, 74.6 years, MMSE 27.3. Majority of subjects were Hispanic or Caucasian. Exclusion criteria were individuals with strong medication, too many health complications. USA	Serum	Microarray	RT-qPCR for serum and also validated using postmortem brain samples	Fold-change analysis showed a significant upregulation of serum miR-455-3p in AD compared to MCI and NC. Expression of serum miR-4668-5p was also significantly upregulated in MCI compared with NC, but was not increased in AD. Validation also performed with frontal cortex of postmortem brain samples. A significant upregulation of miR-455-3p expression was found for AD Braak stage V compared with NC. Similarly expression of miR-3613-3p was significantly higher in AD Braak stage V compared with NC. Expression of miR-4674 was significantly higher in AD Braak stage VI compared to NC.	ROC indicates serum miR-455-3p is a fair test to distinguish AD from NC (AUC 0.79). Also miR-455-3p in postmortem brain is a good test to distinguish AD from NC (AUC 0.86).	Serum miR-455-3p may be a potential biomarker for AD.

Table 2 Continued

Reference	No. of patients, gender, age, ethnicity/ country, inclusion/exclusion criteria	Sample for assay	Screening/discovery method	Validation method	Dysregulated miRNAs	Functional outcomes, receiver operating characteristics (ROC) analysis, specificity/sensitivity	Conclusion, comments
Hara et al. (2017)	NIG validation set: AD, 13M/23F, 74.7 years, MMSE 19.3; NC, 4M/18F, 73.7 years, MMSE 29.3. Japan	Serum with minimal hemolysis	Next-generation sequencing (NGS). Screening/ discovery set, ROW, consisted of samples from JBBNNR, Japan, with serum collected from donors within 2 weeks before death.	RT-qPCR	In NIG validation, expression of miR-501-3p was significantly decreased in AD compared with NC and significantly correlated with MMSE scores. MiR-26b-5p showed marginal significant increase in AD compared with NC (P = 0.066). In brains of AD in ROW set, miR-501-3p was markedly upregulated and still had a significant change in expression level after adjusting for age, gender, APOE genotype, RNA integrity number (RIN).	ROC shows serum miR-501-3p is a good test to distinguish AD from NC (AUC 0.82, sensitivity 0.53, specificity 1.00). MiR-501-3p levels in the brain significantly correlated with Braak NFT stages. As AD pathology progressed, brain mR-501-3p levels increased while serum levels decreased.	MiR-501-3p is dysregulated in the brain as well as in serum of the same individuals during AD progression.
Jia et al. (2016)	AD, 62M/22F, 81.4 years, MMSE 18.3; NC, 41M/21F, 76.2 years, MMSE ND. Probable AD underwent clinical evaluation, magnetic resonance imaging, neuropsychological testing. All NC either diagnosed without neurological disorder or with a systemic disease without neurological manifestations. China	Serum - was separated by centrifugation, followed by 30 minutes of clotting at room temperature	PCR	-	A statistically significant decrease in serum miR-29, -125b, -223, but an increase in miR-519 was found for AD compared with NC. Serum miR-223 was positively correlated with MMSE scores. Dividing patients into 3 groups based on MMSE scores (≤ 13, 14–19, ≥ 20), a significant increase in serum miR-223 was observed, suggesting miR-223 nay be a biomarker of AD severity.	ROC indicates serum miR-223 is a fair test for distinguishing AD from NC (AUC 0.786). The combination of serum miR-125b and miR-223 gives improved sensitivity/specificity (AUC 0.879) which is 15.3% higher than that of serum miR-125b alone and 9.3% higher than that of serum miR-223 alone.	MiR-223 may be a potential biomarker in AD
Blood plasma							
Nagaraj et al. (2017)	AD, 10M/10F, 69.7 years, MMSE 20.5, cerebrospinal fluid (CSF) Aβ42 390 pg/mL, t-tau 767 pg/mL; MCI, 7M/8F, 65.1 years, MMSE 25.9, CSF Aβ42 627 pg/mL, t-tau 450 pg/mL; NC, 15, age-matched. Poland	Plasma without hemolysis and blood cells	RT-qPCR	RT-qPCR	32 microRNAs differentiated AD from NC. Subsequent statistical analysis identified 13 as candidates for validation stage including 2 more microRNAs. Increased levels of miR-486-5p and miR-483-5p were the most significant indicators of MCI and AD. Also upregulation of miR-502-3p and miR-200a-3p in MCI and AD compared to NC was observed.	ROC indicates that miR-483-5p and miR-502-3p are good tests to distinguish AD from NC, and MCI from NC (AUC > 0.9, specificity and sensitivity > 0.80, repeatedly, in both screening and validation studies).	Based on the highest fold changes, consistent statistical significance of changes, and highest specificity and sensitivity for distinguishing MCI from NC, a set of 6 miRNAs was selected. It comprised 4 upregulated miRNAs (483-5p, 486-5p, 200a-3p, 502-3p) and 2 downregulated miRNAs (30b-5p, 142-3p).
Mendes-Silva et al. (2017)	Major depressive disorder (MDD), 13; MDD with MCI, 11; NC, 19. Brazil	Plasma	Ion proton sequencing	-	10 miRNAs differentially expressed between MDD groups and NC. They were miR-100-5p, -184, -1-3p, -125b-5p, -5572, -let7a-5p, -140-3p, -454-3p, -373-3p, -218-5p. Among these, miR-184 and miR-1-3p were differentially expressed between MDD and MDD with MCI.	-	More specific biomarkers may allow the early recognition of subjects with depression and cognitive impairment, who have an increased risk of developing dementia. This is important for the treatment and prevention of cognitive decline and dementia in the elderly with depression.
Zirnheld et al. (2016)	Mild AD, 9M/7F, 78.2 years, MMSE 23.0; moderate-severe AD, 6M/14F, 77.7 years, MMSE 13.3; MCI, 19M/15F, mean age 78.9 years, MoCA mean score 20.7; NC, 6M/31F, 75.3 years, MMSE 28.7. Probable AD patients with MMSE ≥ 25 and NC < 25 excluded. Canada	Plasma without hemolysis and no cellular ribosomal RNA	NA	RT-qPCR	MiR-34c more highly expressed in moderate-severe and mild AD than in MCI or NC, but no significant difference between moderate-severe AD and mild AD. MiR-181c more abundant in moderate-severe AD than MCI or NC, more abundant in mild AD than MCI and NC, but no difference between moderate-severe and mild AD or between MCI and NC. MiR-411 more highly expressed in moderate-severe and mild AD than in MCI and NC, but no difference between moderate-severe and mild AD or between MCI and NC.	ROC indicates miR-34c is fair test to differentiate moderate-severe AD from MCI (AUC 0.78), good test to distinguish moderate-severe AD from NC (AUC 0.82), fair test to distinguish mild AD from NC (AUC 0.71), and poor test to distinguish mild AD from MCI (AUC 0.68). ROC shows miR-181c differentiates moderate-severe AD from both MCI and NC (AUC 0.86, 0.79, respectively) but a poor test to distinguish between mild AD and MCI or NC (AUC 0.63, 0.60, respectively). ROC shows miR-411 is a good test to distinguish moderate-severe AD from MCI and NC (AUC 0.92, 0.99 respectively, and sensitivity 0.77, 0.77, respectively), and for distinguishing mild AD from MCI and NC (AUC 0.93, 0.98, respectively, and sensitivity 0.79, 0.93, respectively).	MiR-411 is more powerful biomarker than miR-34c or miR-181c to distinguish MCI from mild, moderate and severe stages of AD. Inclusion of MCI cohort is essential in biomarker discovery.

Table 2 Continued

Reference	No. of patients, gender, age, ethnicity/ country, inclusion/exclusion criteria	Sample for assay	Screening/discovery method	Validation method	Dysregulated miRNAs	Functional outcomes, receiver operating characteristics (ROC) analysis, specificity/sensitivity	Conclusion, comments
Kayano et al. (2016)	MCI, 11M/12F, 72.8 years, MMSE 24.3; NC, 12M/18F, 70.4 years, MMSE 28.6. Japan	Plasma	RT-qPCR		Differential correlation analysis was applied to the data set with 85 miRNAs. The 20 pairs of miRNAs which had the difference of correlation coefficients $[r_1 - r_2] > 0.8$ were selected as biomarkers that distinguish MCI from NC.	ROC shows that two miRNA pairs miR-191/miR-101 and miR-103/miR-222 have the highest value AUC 0.962 and are good tests to distinguish MCI from NC. Other miRNA pairs that include miR-191 and miR-125b and miR-590-5p also have a high AUC > 0.95.	Traditional t-test applied to the data set shows AUC by each of the 22 miRNAs has mean value of 0.784 and ranged between 0.748 and 0.828. Differential correlation analysis detected much different and more sensitive MCI markers compared to t- test (differential correlation analysis mean AUC value 0.800). Also highest AUC value of any four miRNAs from the 22 miRNAs was less than the highest in the two-pair approach.
CSF							
Liu et al. (2018)	AD, 8M/9F, mean age 74.1 years, APOE ε4 (positive, %) 76.5; MCI, 19M/17F, 72.4 years, 55.6; Subjective memory complaints (SMC), 21M/20F, 71.6 years, 17.1. China	CSF centrifuged, cell pellet sorted into lymphocyte populations by flow cytometry. Subjects had not recently taken ibuprofen, sulfamethoxazole or other drugs that could increase CSF cells.	RT-PCR	-	Relative expression of let-7b in CSF cells was significantly increased in MCI and AD compared to SMC. Total number of white blood cells was significantly increased in CSF from MCI and AD compared to SMC. Total number of CSF lymphocytes and ratio of $CD4^+$ lymphocytes in MCI and AD were significantly higher than in SMC. Let-7b expression in $CD4^+$ lymphocyte population from MCI and AD was significantly higher than SMC, while let-7b expression in $CD8^+$ T lymphocytes, B lymphocytes and NK cells did not significantly differ. The level of let-7b was significantly correlated with CSF t-tau and p-tau but not Aβ40 or Aβ42.	ROC shows probability of occurrence of AD using Aβ40 and Aβ42 are 69.0% and 72.4%, respectively, with AUC 0.201 and 0.232, respectively. On combining Aβ40 and Aβ42, predicted probability of occurrence of AD is 75.9% with AUC 0.849. On adding let-7b to Aβ40 and Aβ42 as predicted parameters, predicted probability of AD increases to 89.7% with AUC 0.93. For t-tau and p-tau, probability of occurrence of AD is 70.7% and 67.2%, respectively, with AUC 0.726 and 0.843. Combining t-tau and p-tau, probability of occurrence of AD is 72,4% with AUC 0.828. Adding let-7b to t-tau and p-tau as a prediction parameter, predicted probability of AD increases to 91.4% and AUC to 0.920.	Addition of let-7b improves diagnostic performance of Aβ40 and Aβ42, and of t-tau and p-tau.
Riancho et al. (2017)	AD, 6M/22F, mean age 67.7 years, with clinical presentation consistent with AD and positive CSF biomarkers Aβ and tau for AD; NC, 10M/18F, 70.2 years, with no memory impairments and negative CSF biomarkers Aβ and tau for AD. Spain	CSF, raw and exosome enriched. CSF samples were centrifuged. Exosome-enriched CSF obtained using exosome isolation kits.	PCR	RT-qPCR	3 candidates were selected: miR-9-5p, -134, -598 for validation phase using 18 AD and 18 NC CSF samples. MiR-9-5p was detected in 9 of NC samples but not found in any of AD samples. MiR-598 was detected in 13 of NC samples but not found in any of AD samples. No significant differences were found for miR-134 - it was detected in 2 NC and none of AD samples. In exosome-enriched CSF samples, miR-9-5p was detected in 7 out of 10 AD and 3 out of 10 NC samples. MiR-598 was detected in 8 out of 10 AD and 5 out of 10 NC samples. MiR-134 was found in 2 out of 10 AD and 2 out of 10 NC samples.	None of the candidate microRNAs were detected in AD CSF samples, whereas miR-9-5p was present in 50% of NC and miR-598 was in 72% of NC samples. There was a tendency of increased microRNA expression in exosome-enriched AD samples compared with NC, but was not statistically significant.	MiR-9-5p and miR-598 may be potential CSF biomarkers for AD.
Lusardi et al. (2017)	AD, 30M/20F, 69.5 years, MMSE 18.3; NC, 23M/26F, 67.8 years, MMSE 29.2. USA	CSF	RT-qPCR	RT-qPCR	Replicated, high confidence miRNAs increased in AD were miR-378a-3p, -1291, -597-5p, and those decreased in AD were miR-143-3p, -142-3p, -328-3p, -193a-5p, -30a-3p, -19b-3p, -30d-5p, -340-5p, -140-5p, -125b-5p, -26b-5p, -16-5p, -146a-5p, -29a-3p, -195-5p, -15b-5p, -223-3p. Candidate miRNAs not retested increased in AD were miR-520b, -603, -202-3p, -519b-3p, -484, while those decreased in AD were miR-584-5p, -145-3p, -24-3p, -532-5p, -28-3p, -146b-5p, -27b-3p, -331-3p, -145-5p, 590-5p, -365a-3p.	ROC shows top performing linear combinations of 3 and 4 miRNAs have AUC 0.80-0.82 and are a fair test for distinguishing AD from NC.	-

Table 2 Continued

Reference	No. of patients, gender, age, ethnicity/ country, inclusion/exclusion criteria	Sample for assay	Screening/discovery method	Validation method	Dysregulated miRNAs	Functional outcomes, receiver operating characteristics (ROC) analysis, specificity/sensitivity	Conclusion, comments
Muller et al. (2016b)	AD, 26M/31F, 72.9 years, MMSE 20.0; MCI 15M/22F, 73.1 years, MMSE 24.8; NC, 21M/19F, 61.4 years, MMSE 27.2. Patients assessed by clinical and neuropsychological examination, and measurement of CSF Aβ42, t-tau, p-tau. The Netherlands, Italy, Belgium	CSF, AD mean sample storage time 5.6 years, mean No. of leukocytes 1.1/μL; MCI 1.8 years, 0.6/μL; NC 2.5 years, 0.9/μL. No. of erythrocytes was very low.	qPCR	-	After normalization, levels of miR-27a and miR-29a were similar in AD of all centers, whereas differences between AD in levels of miR-125b and miR-146a remained. Levels of miR-29a and miR-146a were higher in non-centrifuged samples compared to centrifuged samples. Comparing AD to NC at The Netherlands centre, the decreased expression of miR-146a and similar levels of miR-27a were confirmed. In contrast to earlier findings, the levels of miR-29a and miR-125b in AD were unchanged. Similar levels of expression of all four miRNAs were found on comparing AD, MCI and NC at all three centers. Increased expression levels of miR-146a in MCI compared to NC were lost when confounding factors (age, gender, sample storage, centrifugation status) were considered. Similarly, increased expression levels of miR-27a, -125b, -146a in MCI compared to AD were lost after correcting for confounding factors.	-	After correcting for confounding factors, no differences in miRNA levels were found between AD, MCI, and NC.
Muller et al. (2016a)	AD, 8M/10F, 70.4 years, MMSE 19.7; NC, 8M/12F, 69.2 years. Patients assessed by clinical and neuropsychological examination, magnetic resonance imaging. Most AD (14/18) had a typical CSF profile of abnormal levels of Aβ42, t-tau, p-tau. The Netherlands	CSF, AD mean no. of erythrocytes 17.7/μL, mean no. of leukocytes 0.6/μL; NC 3.0/μL, 0.4/μL.	qPCR	-	Mean miR-27a levels were similar in AD and NC, but mean miR-29a levels were increased in AD compared to NC and differentiated AD from NC with a sensitivity of 0.78 and specificity of 0.60.	-	-
Brain tissue, olfactory mucosa							
Kumar et al. (2018)	AD, 14M/13F, 79.9 years; NC, 8M/7F, 79.3 years. USA	Frontal cortex Broadmann's Area 10 dissected from brains obtained at postmortem. Banked human skin fibroblasts and lymphoblasts from AD and NC.	RT-PCR	-	By RT-PCR, expression level of miR-455-3p in postmortem brains was significantly higher in AD compared to NC. Expression level of miR-455-3p was significantly higher for fibroblasts and lymphoblasts from sporadic AD compared to NC.	ROC indicates that miR-455-3p level in frontal cortex is a fair test to distinguish AD from NC (AUC 0.792, sensitivity 0.889 and specificity 0.667). Expression level of miR-455-3p for fibroblasts when comparing sporadic AD with NC gives AUC 0.861, sensitivity 0.833, specificity 0.667. Expression level of miR-455-3p for B-lymphoblasts when comparing sporadic AD with NC gives AUC 0.722, sensitivity 0.667, specificity 0.500. For postmortem brains there was a trend of reduced miR-455-3p levels with increased postmortem interval and of increased miR-455-3p levels with patients' age.	MiR-455-3p is a potential biomarker for AD.

Table 2 Continued

Reference	No. of patients, gender, age, ethnicity/ country, inclusion/exclusion criteria	Sample for assay	Screening/discovery method	Validation method	Dysregulated miRNAs	Functional outcomes, receiver operating characteristics (ROC) analysis, specificity/sensitivity	Conclusion, comments
Weinberg et al. (2015)	AD, 4M/6F, 88.6 years, MMSE 17.5, mean PMI 4.8 hours; MCI, 5M/5F, 82.9 years, MMSE 28.0, 5.8 hours; NC, 6M/6F, 83.0 years, MMSE 28.3, 5.7 hours. USA	Frontal and inferior temporal cortex obtained at postmortem. 90% of AD cases, 60% of MCI, 58% of NC classified as Braak stages III–VI.	Microarray	qPCR	By qPCR for frontal cortex, miR-498 and miR-150 were significantly upregulated in AD, and miR-150 was upregulated in MCI, compared to NC. Several miRNAs were significantly downregulated in AD including miR-886-3p, -132, -21, -23a, -140-3p, -212, -23b, let-7d, -181a, -498, -150. Two distinct clusters miR-212/miR-132 and miR-23a/ miR-23b were significantly downregulated in MCI. For temporal cortex, miR-212 and miR-132 expression levels were decreased only in AD, with miR-23a and miR-23b unchanged across the groups.	By qPCR, sirt1 mRNA levels were significantly upregulated by ~40% in frontal cortex of MCI compared to AD and NC, whereas sirt1 levels in temporal cortex were similar across the groups. Increased sirt1 mRNA expression was significantly correlated with decreased miR-212 frontal cortex levels. This association was not found in the temporal cortex.	In vitro studies using human neuronal cells showed that coordinated downregulation of miR-212 and miR-23a increased sirt1 expression and provided neuroprotection from Aβ toxicity. No ROC/AUC analysis was performed to indicate whether the dysregulated miRNAs are good tests to distinguish between AD, MCI and NC.
Moon et al. (2016)	Subjects were categorized based on clinical dementia rating (CDR) score, AD Assessment Scale-Cognitive-Korean version (ADAS-Cog-K) score, and Beck Depression Inventory II (BDI-II) score. CDR 1, 6M/5F, 69 years, MMSE-K Korean version 23.8, ADAS-Cog-K 16.0, BDI-II 9.3; CDR 0.5, 4M/9F, 68 years, 26.8, 11.2, 11.1; CDR 0, 4M/5F, 63 years, 28.3, 8.4, 5.2. South Korea	Olfactory mucosa	RT-PCR	-	MiR-206 was increased in CDR 0.5 and CDR 1 compared to CDR 0 and increased with progression of dementia.	ROC indicates miR-206 is a good test to distinguish CDR 0.5 from CDR 0 (AUC 0.942, sensitivity 0.875 and specificity 0.941 when relative expression of miR-206 over U6 exceeded 7.06×10^{-4}. MiR-206 is a good test to distinguish CDR 1 from CDR 0 (AUC 0.976, sensitivity 0.909 and specificity 0.933 when relative expression of miR-206 over U6 exceeded 49.72×10^{-4}. The diagnostic value of olfactory mucosal miR-206 level was greater for patients <65 years. AUC values for diagnosing CDR 0.5 and CDR 1 dementia were 0.952 and 1.000, respectively. Olfactory mucosal miR-206 level also showed good ability to detect early dementia in patients > 65 years, although with less power than that observed for patients < 65 years.	A significant correlation was found between olfactory mucosal miR-206 level and cognitive function scores.

MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment.

in AD PBMNs compared with NC PBMNs. ROC indicated miR-339 as a fair test to distinguish AD PBMNs from NC PBMNs (AUC 0.768), and miR-425 to be a good test to distinguish AD PBMNs from NC PBMNs (AUC 0.868). MiR-639 was a poor test to distinguish AD PBMNs from NC PBMNs (AUC 0.287). The β-amyloid precursor protein β-secretase BACE1 might be a target of miR-339 and miR-425.

Blood serum

Wu et al. (2017) using RT-qPCR validation with serum samples from 65 AD and 50 NC patients showed that expression levels of miR-146a-5p, -106b-3p, -195-5p, -20b-5p, -497-5p were higher, while those of miR-125b-3p, -29c-3p, -93-5p, -19b-3p were lower in AD than in NC subjects. Computational analysis predicted that 3'-UTR of STAT 3 mRNA was a target of both miR-29c-3p and miR-19b-3p. A large-scale study by Jia et al. (2016) with serum from 84 AD and 62 NC individuals used PCR screening to show a significant decrease in miR-29, -125b, -223 and an increase in miR-519 in AD compared with NC. Serum miR-223 was positively correlated with MMSE scores and might be a biomarker of AD severity. ROC indicated miR-223 to be a fair test to distinguish AD from NC (AUC 0.786). Also the combination miR-223 and miR-125b gave improved sensitivity/specificity (AUC 0.879) than miR-223 or miR-125b alone. Hara et al. (2017) found using a NGS validation set of serum samples from 35 AD and 22 NC patients that miR-501-3p was decreased in AD compared with NC and correlated with MMSE scores. In brains of patients in the screening/discovery set, miR-501-3p was markedly upregulated in AD compared with NC and positively correlated with disease progression as indicated by Braak staging. ROC showed serum miR-501-3p was a good test to distinguish AD from NC (AUC 0.82, sensitivity 0.53, specificity 1.00). A smaller-scale study by Kumar et al. (2017) with serum from 12 AD, 20 MCI and 18 NC subjects using RT-qPCR validation showed a significant upregulation of miR-455-3p in AD compared with MCI and NC. Upregulation of miR-4668-5p occurred for MCI compared with NC but not AD. Using frontal cortex of brain postmortem samples, a significant upregulation of miR-455-3p and miR-3613-3p was observed for AD Braak stage V compared with NC, and also of miR-4674 in AD Braak stage VI compared with NC. ROC indicated serum miR-455-3p to be a fair test to distinguish AD from NC (AUC 0.79), and miR-455-3p in postmortem brain to be a good test to distinguish AD from NC (AUC 0.86).

Blood plasma

Zirnheld et al. (2016) analysed blood plasma from 20 moderate-severe AD, 16 mild AD, 34 MCI and 37 NC subjects by RT-qPCR validation and found miR-34c was more highly expressed in moderate-severe and mild AD than in MCI or NC, but with no significant difference beween moderate-severe and mild AD or between MCI and NC. In addition, miR-181c was more abundant in moderate-severe and mild AD than MCI or NC, but no difference between moderate-severe and mild AD or between MCI and NC. MiR-411 was more highly expressed in moderate-severe and mild AD than in MCI or NC, but no difference between moderate-severe and mild AD or between MCI and NC. ROC indicated miR-34c to be a fair test to differentiate moderate-severe AD from MCI (AUC 0.78), a good test to distinguish moderate-severe AD from NC (AUC 0.82), and a poor test to distingush MCI from NC (AUC 0.68). Similarly, miR-181c differentiated moderate-severe AD from both MCI and NC (AUC 0.86, 0.79, respectively) but was a poor test to distingiush between mild AD and MCI or NC (AUC 0.63, 0.60, respectively). MiR-411 was a good test to distinguish moderate-severe AD from MCI and NC (AUC 0.92, 0.99, respectively, and sensitivity 0.77, 0.77 respectively) and for distinguishing mild AD from MCI and NC (AUC 0.93, 0.98, respectively, and sensitivity 0.79, 0.93, respectively). Hence miR-411 was a more powerful biomarker than miR-34c or miR-181c to distinguish MCI from mild, moderate and severe AD. Kayano et al. (2016) used RT-qPCR screening of microRNAs in blood plasma of 23 MCI and 30 NC subjects. Differential corelation analysis identified 20 pairs of microRNAs that distinguish MCI from NC. ROC showed that two pairs miR-191/miR-101 and miR-103/miR-222 had the highest AUC 0.962, and were good tests to distinguish MCI from NC. Other microRNA pairs that included miR-191 and miR-125b and miR-590-5p also had high AUC > 0.95. Differential correlation analysis detected much different and more sensitive biomarkers for MCI than *t*-test (mean AUC 0.800). Nagaraj et al. (2017) performed RT-qPCR validation with blood plasma from 20 AD, 15 MCI and 15 NC and found that increased levels of miR-486-5p and miR-483-5p were the most significant indicators of MCI and AD. In addition, there was upregulation of miR-502-3p and miR-200a-3p in MCI and AD compared with NC. ROC indicated that miR-483-5p and miR-502-3p were good tests to distinguish AD from NC, and MCI from NC (AUC > 0.9, specificity and sensitivity > 0.80). A set of 6 microRNAs was selected for distingushing MCI from NC and comprised 4 upregulated microRNAs (483-5p, 486-5p, 200a-3p, 502-3p) and 2 downregulated microRNAs (30b-5p, 142-3p). For MCI and AD patients, diagnoses were confirmed by CSF biomarkers. Mendes-Silva et al. (2017) performed microRNA screening of blood plasma from 13 MDD, 11 MDD with MCI and 19 NC individuals using ion proton sequencing. Ten microRNAs were differentially expressed between MDD and NC, among which miR-184 and miR-1-3p were differentially expressed between MDD and MDD with MCI.

CSF

Lusardi et al. (2017) performed RT-qPCR validation of microRNAs in CSF from 50 AD and 49 NC subjects and found that replicated microRNAs increased in AD were miR-378a-3p, -1291, -597-5p, and those decreased in AD were miR-143-3p, -142-3p, -328-3p,-193a-5p, -30a-3p, -19b-3p, -30d-5p, -340-5p, -140-5p, -125b-5p, -26b-5p, -16-5p, -146a-5p, -29a-3p, -195-5p, -15b-5p, -223-3p. Candidate miRNAs not retested increased in AD were miR-520b, -603, -202-3p, -519b-3p, -484, while those decreased in AD were miR-584-5p, -145-3p, -24-3p, -532-5p, -28-3p, -146b-5p, -27b-3p, -331-3p, -145-5p, 590-5p, -365a-3p. ROC indicat-

ed that the top performing linear combinations of 3 or 4 microRNAs were a fair test to distinguish AD from NC (AUC 0.80–0.82). Muller et al. (2016b) also used large group sizes of 57 AD, 37 MCI and 40 NC individuals to perform qPCR screening of microRNAs in CSF samples collected at three different centers. Importantly, no differences in microRNAs levels were found between AD, MCI and NC after correcting for confounding factors that included age, gender, sample storage time, centrifugation status. In a separate study, Muller et al. (2016a) showed using qPCR screening of microRNAs in CSF samples from 18 AD and 20 NC subjects with low concentrations of erythrocytes and leukocytes that miR-29a was upregulated in AD compared with NC and differentiated AD from NC with a sensitivity of 0.78 and specificity of 0.60. Riancho et al. (2017) performed RT-qPCR validation of microRNAs in raw and exosome-enriched CSF samples from 28 AD and 28 NC individuals. AD patients had clinical presentation consistent with AD and positive CSF biomarkers Aβ and tau for AD. NC subjects had no memory impairments and negative CSF biomarkers Aβ and tau for AD. MiR-9-5p was not detected in AD but was present in 50% of NC samples. Similarly, miR-598 was absent in all AD but present in 72% of NC samples. Liu et al. (2018) collected CSF from 17 AD, 36 MCI and 41 SMC (subjective memory complaints) patients and which was centrifuged and cell pellet sorted into lymphocyte populations by flow cytometry. RT-PCR screening of microRNAs showed the relative expression of let-7b in CSF cells was significantly increased in AD and MCI compared with SMC. Total number of CSF lymphocytes and ratio of CD4+ lymphocytes in AD and MCI were significantly higher than in SMC. Let-7b expression in CD4+ lymphocytes from AD and MCI was significantly higher than SMC, while let-7b expression in CD8+ T lymphocytes, B lymphocytes and NK cells were not significantly different. Adding let-7b to Aβ40 and Aβ42 as predictive parameters increased the probability of predicting AD to 89.7% (AUC 0.93).

Brain tissue and olfactory mucosa

Kumar and Reddy (2018) using RT-PCR screening of microRNAs in frontal cortex Broadmann's Area 10 of postmortem brains of 27 AD and 15 NC individuals found that the expression of miR-455-3p was significantly increased in AD compared with NC. Also using banked skin fibroblasts and lymphocytes from AD and NC individuals, expression of miR-455-3p was significantly higher for AD compared with NC. ROC indicated that frontal cortex miR-455-3p was a fair test to distinguish AD from NC (AUC 0.792, sensitivity 0.889, specificity 0.667). Weinberg et al. (2015) performed qPCR validation of microRNAs in frontal and inferior temporal cortex of postmortem brains of 10 AD, 10 MCI and 12 NC subjects. In frontal cortex, miR-498 and miR-150 were significantly upregulated in AD, and miR-150 was upregulated in MCI, compared with NC. Several microRNAs were significantly downregulated in AD including miR-886-3p, -132, -21, -23a, -140-3p, -212, -23b, let-7d, -181a, -498, -150. Two distinct clusters miR-212/miR-132 and miR-23a/miR-23b were significantly downregulated in MCI. For temporal cortex, miR-212 and miR-132 expression levels were decreased only in AD, with miR-23a and miR-23b being unchanged across the groups. Moon et al. (2016) used RT-PCR screening of microRNAs in olfactory mucosa of subjects categorized on clinical dementia rating (CDR) score, ADAS-Cog-K score and Beck Depression Inventory II score. The groupings were 11 CDR 1, 13 CDR 0.5, and 9 CDR 0. Olfactory mucosa miR-206 was increased in CDR 0.5 and CDR 1 compared with CDR 0 and increased with progression of dementia. ROC indicated miR-206 was a good test to distinguish CDR 0.5 from CDR 0 (AUC 0.942, sensitivity 0.875, specificity 0.942). Also it was a good test to distinguish CDR 1 from CDR 0 (AUC 0.976, sensitivity 0.909, specificity 0.933).

The microRNAs indicated to have upregulated or downregulated expression in blood serum, blood plasma, CSF of AD and MCI patients are shown in **Figure 1**.

Future Perspectives

The development of reliable, minimally or non-invasive methods for diagnosing early AD in MCI patients is imperative for increased efficiency of existing therapies, as well as for recruiting patients for clinical trials of new drugs, monitoring disease progression and response to treatment. At present the most reliable validation of blood or CSF microRNAs as biomarkers for AD is by histopathologic examination of postmortem brain sections. No methods are available to evaluate the expression of a specific microRNA in the brain of a living person. The olfactory epithelium has been shown to reflect brain Aβ and tau pathology in the majority of cases with pathologically verified AD (Arnold et al.,

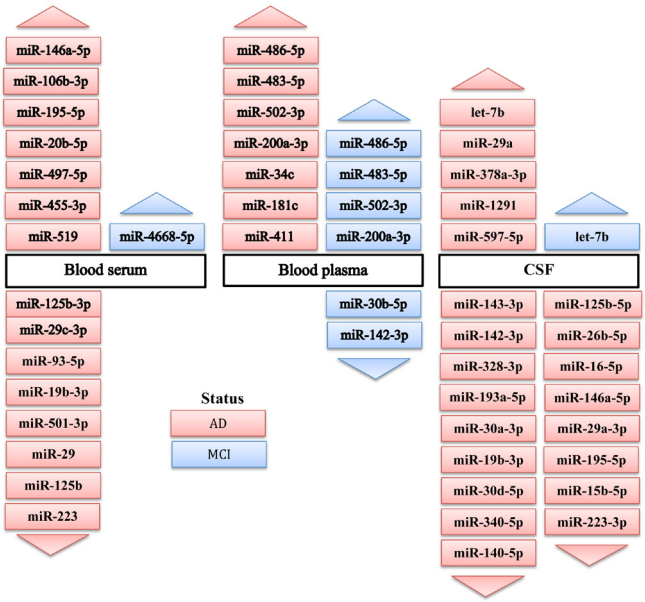

Figure 1 Changes in microRNA expression in blood serum, blood plasma, and cerebrospinal fluid (CSF) of Alzheimer's disease (AD) and mild cognitive impairment (MCI) patients compared to normal controls as indicated by polymerase chain reaction/real-time polymerase chain reaction (PCR/RT-PCR) screening and validation assays in the articles reviewed.

Increased or decreased expression is indicated by arrows pointing upwards or downwards, respectively. Color code is AD (red) and MCI (blue).

2010). Measurement of microRNAs in olfactory epithelium could be performed in patients with CSF levels of Aβ and tau indicative of AD as a possible way of validating changes in blood microRNA levels. In this regard, the olfactory epithelium miR-206 level was significantly correlated with cognitive assessment score in non-depressed subjects with cognitive impairment and could serve as a diagnostic marker of early AD (Moon et al., 2016).

Histomicroscopic examination of brain tissue specimens enables classification into early-, mid- and late-stages of AD. Brains stored in brain banks could be used for this purpose and blood collected from patients shortly before their death could be examined at early-stage AD (Braak NFT stages I and II) for diagnostic biomarkers. In addition progression of the disease could be monitored through biomarker analysis using blood samples from patients at mid-stage AD (Braak NFT stages III and IV) and late-stage AD (Braak NFT stages V and VI). Kumar et al. (2017) showed a gradual increase or decrease in several chosen serum microRNAs for controls, MCI and AD patients and could be used to monitor disease progression to AD. Also Hara et al. (2017) found in brains of patients in the screening/discovery set, miR-501-3p was markedly upregulated in AD compared with NC and positively correlated with disease progression as indicated by Braak staging. Keller et al. (2016) replicated and validated previous findings in a US study of blood microRNAs in AD patients compared to controls with a new cohort of subjects in Germany using next-generation sequencing. Included in the validation cohort were MCI patients and a substantial dysregulation of microRNAs in AD patients was found compared to MCI with 119 being upregulated and 29 downregulated in AD compared to MCI. Upregulated expression of miR-151a-3p and downregulated expression of miR-17-3p was found for AD compared with NC. In addition, blood samples of AD patients with disease duration < 2 years (early-stage) were compared to those of patients with longer disease duration (mid-/late-stage) and no significant differences in microRNAs were observed. (For time frame of the stages in AD progression see Ellis and Higuera, 2016). These findings warant further replication using larger-sized cohorts and RT-qPCR validation.

Several of the reviewed studies showed that microRNAs could distinguish MCI from NC subjects. For example, Kumar et al. (2017) observed an upregulation of miR-4668-5p in blood serum for MCI compared with NC but not AD patients. Kayano et al. (2016) using differential corelation analysis identified 20 pairs of microRNAs in blood plasma that distinguish MCI from NC, and these included the two pairs miR-191/miR-101 and miR-103/miR-222. Differential correlation analysis detected much different and more sensitive MCI biomarkers compared to *t*-test. Also the highest AUC value of any four microRNAs was less than the highest in the two-pair approach. Nagaraj et al. (2017) reported a set of 6 microRNAs in blood plasma that distingushed MCI from NC, and which was comprised of 4 upregulated microRNAs (483-5p, 486-5p, 200a-3p, 502-3p) and 2 downregulated microRNAs (30b-5p, 142-3p). Liu et al. (2018) showed that let-7b expression in $CD4^+$ lymphocytes isolated from CSF cell pellets of AD and MCI individuals was significantly higher than for SMC (subjective memory complaints) patients.

It is recognized that MCI patients are the most promising group of patients for whom therapy could be initiated to delay the onset of AD (Michael-Titus et al., 2010). Identification of microRNAs that differentiate MCI or AD from NC with high sensitivity and specificity also provides a feasible therapeutic strategy. MicroRNA mimics or agomirs could be administered to increase the levels of specific microRNAs that are downregulated, while microRNA inhibitors or antagomirs could be given to lower the levels of those microRNAs that are upregulated, in MCI or AD compared to NC. These agents should be tested in future clinical trials. An increased risk of developing AD has been reported for females compared with males at age of 65–75 years (Neu et al., 2017). Many of the studies presented in this review had considerable disparity in the numbers and proportions of male and female subjects in the AD, MCI and NC groups, as well as significant variation in age and extent of disease progression.

A panel of microRNAs has a higher AUC value than a single microRNA for distinguishing AD from NC (Jia et al., 2016). Nagaraj et al. (2017) reported a 6 microRNA panel in blood plasma which distinguished non-demented control subjects from MCI patients diagnosed with probable early AD by CSF assay of Aβ42, total tau and phosphorylated tau. These 6 microRNAs were functionally mapped to proteins involved in AD pathology by searches in databases containing predictive and validated microRNA targets including β-secretase 1 (BACE1), BACE2, microtubule-associated protein/tau, and presenilin 2 (PSEN2). It was suggested that the 6 microRNA panel might serve as a possible replacement of invasive CSF biomarkers to identify early AD (Najaraj et al., 2017). Such a microRNA panel together with correlation analysis of specific paired microRNAs would provide a very good test for differentiaing early AD from NC. The amyloid precursor protein (APP) cleavage enzyme BACE1 was also a possible direct target of miR-425-5p and miR-339-5p which were upregulated in PBMNs from AD compared to NC group. Overexpression of miR-425-5p decreased BACE1 protein levels (Ren et al. 2016).

A number of differentially expressed genes were found to be regulated by microRNAs. These included *PLCB2*, *CDK5R1*, *LRP1*, *NDUFA4*, *DLG4* which were regulated by miR-26b-5p, -103a-3p, -107. -26a-5p and these microRNAs had increased expression levels in blood samples of AD compared with NC (Chang et al., 2017). Also 14 validated target genes were found of at least 5 of 33 microRNAs overalapping in screening and validation studies of blood samples of AD compared with NC and were *VEGFA*, *DICER1*, *AGO1*, *PTEN*, *CDKN1A*, *APP*, *RBI*, *CCND1*, *CCND2*, *WEE1, IL13*, *HMGA2, TNFRSF1OB, MYC* (Keller et al., 2016). Many of these have key roles in AD and microRNAs might regulate the genes involved in signaling pathways. For example, low serum levels of vascular endothelial growth factor (VEGF) were associated with AD (Mateo et al., 2007) and VEGF was found to be expressed in the brains of AD patients and to increase with AD severity (Thomas et al., 2015). Also the tumor-suppressor phosphatase and tensin homolog (PTEN)

was found to accumulate in NFTs (Sonoda et al., 2010). PTEN affects tau phosphorylation, binding to microtubules and formation of aggregates and neurite outgrowth (Zhang et al., 2006). PTEN is a negative regulator of PI3 kinase and the predominant effects on tau appeared to be medited by reducing ERK1/2 activity (Kerr et al., 2006). Furthermore, the expression of miR-455-3p was upregulated in blood serum of AD compared to NC and shown to have a relationship with 11 biological pathways and associated genes. The most important signaling pathways were extracellular matrix (ECM)-receptor interaction, adherence junction, transforming growth factor-β (TGF-β) signaling pathway, hippo signaling pathway, cell cycle pathway, and the regulation of the actin cytoskeleton. The upregulation of miR-455-3p in AD development might be associated with these signaling pathways and through altered expression of *HSPG2*, *THBS1*, *COL3A1*, *COL6A1*, *TNC*, *MYC*, *Smad2*, *RAN*, *PLK1*, *TP73*, *ACTN1* and *IQGAP1* genes (Kumar et al., 2017). Computational analysis predicted that the 3'-UTR of signal transduction and activator of transcription 3 (STAT3) mRNA to be a target of miR-29c-3p and miR-19b-3p, both of which had lower serum expression levels in AD than in NC subjects. A regulatory network of microRNAs and target genes was identified and contained miR-29c-3p and miR-19b-3p, 4 AD virulence genes, and STAT3 (Wu et al., 2017). Several studies have suggested that STAT3 activation can promote glial differentiation from neural progenitor cells and inhibit neuronal differentiation of neural progenitor cells (Choi et al., 2003; Sriram et al., 2004; Okada et al., 2006). STAT3 can cause excessive gliosis (Kwak et al., 2010; Tsuda et al., 2011) which is often found in AD patients. Interestingly, miR-501-3p had lower serum expression level in AD compared to NC but was upregulated in the brains of AD patients (Hara et al., 2017). It is possible that miR-501-3p upregulation could cause alterations in the cell cyle of AD brains. Inappropriate cell cycle re-entry in postmitotic neurons, which leads to apoptotic death, is an early sign that preceeds the formation of amyloid plaques and NFTs (Kruman et al., 2004; Borda et al., 2010; Swerdlow, 2012). Alternatively, miR-501-3p could mediate the activity-dependent regulation of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate) receptor subunit GluA1 in dendrites, suggesting that it contributes to synaptic plasticity related to cognitive functions, including learning and memory (Hu et al., 2015). In the differential correlation analysis study, miR-125b was one of the plasma microRNAs found in several pairs having a high AUC value (≥ 0.95) for MCI detection (Keller et al., 2016). Also miR-125b was dowregulated in serum of AD compared to NC (Wu et al., 2017). MiR-125b was shown to bind to 3′-UTR of p53 mRNA and worked as a negative regulator of p53 (Le et al., 2009). p53 has been explored as a tumor supressor but other aspects have been reported including to control diseases, aging and metabolism (Vousden and Lane, 2007). p53 promotes apoptosis and has important implications for the brain during neurodegeneration in AD (Perluigi et al., 2016). MAPK, TGF-β and neurotrophin signaling pathways were indicated as characteristic in MCI. Similarly to p53 signaling, these pathways have common biological functions such as cell survival, cell cycle and apoptosis (Kayano et al., 2016). MAPK pathway is also known to be involved in aberrant cellular signaling in AD pathology (Schnoder et al., 2016). Finally, animal models of AD and MCI have been described and could be used to examine and verify microRNA profiles in blood, blood serum, blood plasma and brain tissues and to trial microRNA-based therapies. Transgenic mice overproducing mutant APP were found to develop extracellular Aβ plaques which was age-dependent, only occurring in mid to late adulthood in most of the animals (LaFeria and Green, 2012). While these mice did not develop NFTs, many exhibited increased tau hyperphosphorylation (Gotz J et al., 2007). These animals develop marked cognitive decline and undergo subtle alterations in tau that resemble the precursors to NFTs in the human brain (LaFeria and Green, 2012). Transgenic mice that express further gene alterations in addition to mutated APP such as mutated human tau (Lewis et al., 2001; Oddo et al., 2003) or removal of nitric oxide synthase 2 (Wilcock et al., 2008) develop NFTs similar to those in the human AD brain. One of the main considerations is that AD mouse models do not show the extensive neuronal loss found in the brains of AD patients. Most of the AD patients at clinical diagnosis already have reached Braak stage V or VI with marked synaptic and neuronal loss. Moreover, the loss of synapses is the best correlate of cognitive impairment in patients with AD (DeKosky et al., 1996). The synapse loss, which occurs before neuronal death in humans, is present in most of the mouse models and gives rise to the memory deficits seen in behavioral tasks for testing memory function. The memory deficits can be associated with neuropathological alterations (Pepeu, 2004). Hence, therapies for reversing memory deficits in AD mouse models might aid in treating the memory decline in patients with MCI (Cuadrado-Tejedor and Garcia-Osta, 2014). The appearance of amyloid plaques and synapse loss appears in some mouse AD models even at 2 to 4 months of age. Aging is the most important risk factor for AD and despite being such an important risk factor it is often absent in studies with animal models. Therefore the use of late-plaque models for preclinical studies (*e.g.*, Tg2576, PDAPP, TgAPP23) could be more appropriate than using early-plaque models (Lee and Han, 2013). In addition, late-plaque and early-plaque models may provide complementary data necessary to decipher the role of microRNAs as diagnostic and therapeutic tools for AD. In summary, considerable advances have been made in the recent studies included in this review with regard to distinguishing MCI and AD from NC by analyzing microRNAs in blood serum, blood plasma and CSF (**Figure 1**), and have included individual and combinations of microRNAs as well as differential correlation of paired microRNA testing. Limitations that were identified in previous studies (**Table 1**) included small group sizes, and marked disparity of individuals in the AD, MCI and NC groups including age, gender, number, ethnicity, stage of disease progression, screening and validation methods, data processing and normalization, statistical analysis. These have been taken into consideration in many of these recent studies, but some concerns still re-

main regarding recruitment of patients including numbers, gender, inclusion and exclusion criteria, medications taken by the patients, most appropriate validation methods, normalization and statistical analysis of data. It is hoped that future studies will continue to address these concerns in the planning and implementing of such studies so that a sensitive and specific, minimally invasive test can be developed for identifying patients with MCI (early AD) and therapy initiated to slow the memory decline and progression to AD.

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