Article

Differential Expression of MicroRNAs in Alzheimer's Disease: A Systematic Review and Meta-analysis

Running title: MiRNAs in Alzheimer's Disease: A Meta-analysis

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Keywords: Dementia; Alzheimer's disease; MicroRNAs; Biomarker; Diagnosis; Meta-analysis

ABSTRACT

Alzheimer's disease (AD) results in progressive cognitive decline owing to the accumulation of amyloid plaques and hyperphosphorylated tau. MicroRNAs (miRNAs) have attracted attention as a putative diagnostic and therapeutic target for neurodegenerative diseases. However, existing meta-analyses on AD and its association with miRNAs have produced inconsistent results. The primary objective of this study is to evaluate the magnitude and consistency of differences in miRNA levels between AD patients, mild cognitive impairment (MCI) patients and healthy controls (HC). Articles investigating miRNA levels in blood, brain tissue, or cerebrospinal fluid (CSF) of AD and MCI patients versus HC were systematically searched in PubMed/Medline from inception to February 16th, 2021. Fixed- and random-effects meta-analyses were complemented with the I^2 statistic to measure the heterogeneity, assessment of publication bias, sensitivity subgroup analyses (AD severity, brain region, post-mortem versus ante-mortem specimen for CSF and type of analysis used to quantify miRNA) and functional enrichment pathway analysis. Of the 1,512 miRNAs included in 61 articles, 425 meta-analyses were performed on 334 miRNAs. Fifty-six miRNAs were significantly upregulated (n=40) or downregulated (n=16) in AD versus HC and all five miRNAs were significantly upregulated in MCI versus HC. Functional enrichment analysis confirmed that pathways related to apoptosis, immune response and inflammation were statistically enriched with upregulated pathways in participants with AD relative to HC. This study confirms that miRNAs' expression is altered in AD and MCI compared to HC. These findings open new diagnostic and therapeutic perspectives for this disorder.

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disorder and the leading cause of dementia,[1, 2] with substantial socioeconomic costs in aging populations,[3] but its etiology is still poorly understood.[4] Previous studies have reported amyloid beta (Aβ) peptides and hyperphosphorylated tau as key proteins in the pathophysiology of the disease. However, multicenter clinical trials targeting Aβ have not been successful,[5] and the outcome of anti-tau therapy is still unclear.[6] Therefore, it is imperative to better understand the etiology of AD to further inform innovative therapeutic interventions.

Noncoding RNAs (ncRNAs) are significant regulators orchestrating gene expression.[7] Growing evidence suggests that various regulatory ncRNAs, such as long noncoding RNAs (lncRNAs) and microRNAs (miRNAs), play an important role in the onset and development of neurodegenerative diseases such as neuronal differentiation and synaptic plasticity.[8, 9] MiRNA participates in negative gene expression regulation post-transcriptionally[10, 11] and is involved in neuropathological mechanisms such as neurogenesis and nerve cell apoptosis.[12] Thus, regulation of miRNAs regulating the AD-related gene ADAM10, and BACE1 expression are indicative of the risk of developing AD.[13] MiRNA dysregulation has been confirmed in *in vitro* and animal models. The reduction of miR-298 and miR-328 was associated with higher BACE1 in a cellular AD model.[14] It was also found that mice with a significantly increased miR-124 level in the hippocampus had significant memory dysfunction.[15]

Despite the substantial interest in this field, the results of miRNA studies that have examined plasma, cerebrospinal fluid (CSF), or brain miRNA concentrations in AD have been inconsistent. For example, one study reported upregulation of hsa-miR-125b-5p[16] while

another reported downregulation of the same miRNA in CSF.[17] Furthermore, there are only a few available comprehensive systematic reviews and meta-analyses focusing on miRNA.[13, 18]

The primary objective of this study is to close this knowledge gap and test the consistency and magnitude of putative differences in all miRNAs between AD and mild cognitive impairment (MCI) patients and healthy controls (HC). In the current study, we conducted a systematic review and meta-analysis of miRNA concentrations in brain, blood, or CSF of AD and MCI patients compared to those of HC. Through this study, we will provide a summary of the evidence in this field to advance clinical knowledge. Therefore, we aim to identify target miRNAs which can be used as a powerful diagnostic tool and in the development of miRNA-based drugs.

METHODS AND MATERIALS

Search strategy and study selection

The present study protocol was registered in PROSPERO (registration: CRD42020150993).[19] Two researchers (SY and SEK) independently conducted a systematic PubMed/Medline search using the following search terms: "(noncoding RNA OR non-coding RNA OR ncRNA OR microRNA OR miRNA OR miR OR micro-RNA) AND (Alzheimer* or MCI or (mild cognitive impairment))". The literature search was performed from inception to 16th February 2021 with an English-language restriction. Original clinical studies with no study design restrictions that reported means and standard deviations or associated measures on blood, CSF, or brain concentrations of isolated miRNA in patients with AD or MCI and HC were included. There was no limitation on the cohort size, gender, and age. Exclusion criteria were (1) cell-based and nonhuman studies; (2) genetic and familial AD studies; (3) samples overlapping with other studies. The meta-analyses performed in this study followed the guidelines recommended in the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-analysis, **Supplementary Table 1**).[20] An additional description of all the methods applied in this study is provided in the Supplementary Material.

Data extraction

To avoid potential errors, two authors (SY and SEK) independently extracted data from eligible studies. Data on the number of patients, number of controls, mean miRNA concentration, and standard deviations were extracted as primary outcomes. Discrepancies in data entry were double-checked and inconsistencies were resolved through discussion with other authors and consensus was reached. Authors were contacted when data were not

available. The first author's name, publication year, PubMed identifier (PMID), city of origin, miRNA identifiers, specimen source, specific details of brain specimen type, diagnostic criteria, patient characteristics, experimental methods (i.e., quantitative real-time polymerase chain reaction (RT-qPCR), deep sequencing) and housekeeping gene used for normalization were collected and entered into a local database.

Quality assessment

The Newcastle-Ottawa Scale (NOS) for case-control studies, as recommended by the Cochrane Collaboration, was used to assess the quality of the eligible observational studies.[21, 22] The scale uses a star system for the number of criteria met.

Statistical analysis

All statistical analyses were performed using Comprehensive Meta-analysis version 3.3.070 software (Biostat, Englewood, NJ, USA). For quality control, miRNAs with different names in different publications were converted to miRbase, v21 (http://www.mirbase.org) reporting established miRNA sequence identifiers. In this study, $p < 0.05$ values were considered as being statistically significant.

Functional enrichment analysis

A miRNA literature search was performed on individual studies, review articles, and NCBI Gene (http://www.ncbi.nlm.nih.gov/gene) to identify functions of meta-analyzed miRNAs. Since we included miRNAs from three specimen sources: blood, brain and CSF, we used a

miRNA set enrichment tool, TAM2.0, for functional enrichment analysis to identify enriched pathways.[23] For functional enrichment, we selected the top 60 miRNAs from three types of origin and identified the enriched functional pathways (p-value < 0.05) associated with these miRNAs (60 miRNAs for brain and CSF, 33 for blood since only 33 blood miRNAs were meta-analyzed). We also performed the functional enrichment analysis based on up-/down-miRNAs from three origins and extracted commonly enriched pathways.

RESULTS

Search strategy and selection criteria

The initial literature search returned 2,744 potentially eligible articles (**Figure 1**). Of these, 2,407 were excluded based on title and abstract screening, leaving 337 studies for further evaluation. Among them, 61 original studies fulfilled the inclusion criteria and were included in the meta-analysis. The reasons for the papers excluded through full-text screening are detailed in **Figure 1**.

Study characteristics

Supplementary Table 2 summarizes the included studies. Briefly, 6,853 participants, consisting of 3,442 subjects with AD, 302 subjects with MCI and 3,109 HC were included (**Table 1**). Of these, 465 AD patients and 406 HC were included in more than one analysis.

In total 3,560 data were extracted from the included studies. Among them, 26 (0.73%) were for MCI and 3,534 (99.27%) were for AD. Of these, 406 miRNAs were found in two specimens and 60 miRNAs were found in all three specimens. A total of 1,188 data (33.37%) were included in the meta-analysis since data from more than 2 articles are required to run the meta-analysis. A total of 425 miRNAs were meta-analyzed, of which 5 were for MCI and the remaining 420 were for AD (**Table 1**).

For the between-group meta-analysis, 1,001 data evaluated the miRNA concentration with PCR (84.54%) and 183 with deep sequencing methods (15.46%). Two studies with 175 data assessed miRNA concentrations in severe AD patients with Braak stage VI, and 3 studies with 13 data in mild AD with Braak stage III-IV. Specific brain regions in the meta-analyses

consisted of 167 temporal cortex, 40 hippocampus, and 8 prefrontal cortex data. Of 851 CSF meta-analyzed data, 436 were obtained from living patients (51.23%) and 27 were from post-mortem patients (3.17%).

The quality of studies and risk of bias were measured using the NOS and ranged from 2 to 9. All studies were case-control studies, with a mean score of 5.57, and we considered a study which scored >6 a high-quality, and <3 a low-quality study, since a reference value for high quality studies has not been established yet (**Supplementary Table 3**).

Meta-analysis results

Meta-analysis for all patients. A summary of the overall meta-analysis results is shown in **Table 1**. All meta-analysis results are listed in **Supplementary Tables 4-10**. Among 19 miRNAs with significant differential expression in blood specimen, 5 were analyzed only by PCR (**Supplementary Table 4**). There was one lncRNA, BACE1-AS, in blood but the expression difference was not significant (**Supplementary Table 5**). In brain specimen, among 8 miRNAs with significant differential expression, miR-2476 and miR-1386 were derived from sequencing data only (**Supplementary Table 6**). There were 291 miRNAs detected in CSF, with 29 significant results (**Supplementary Table 8, 9**). MiRNAs with significant effect size difference are summarized in **Table 1**. Among the meta-analyses, 61 (14.35%) miRNAs had significantly differential expression (p-value < 0.05), 5 of them were for MCI (100%) and remaining 56 were for AD (13.18%) (**Table 1**). All 5 miRNAs with MCI had p-values of less than 0.001 while there were 8 (1.90%) with AD (**Figure 2**). To overcome a large heterogeneity of contributing studies, we conducted subgroup analysis.

Subgroup analysis. Of the 19 and 62 miRNAs meta-analyzed in brain with PCR and

sequencing data, 9 (47.37%) and 2 (3.23%) miRNAs yielded were differentially expressed in AD and HC, respectively. Among the 66 meta-analyses with severe AD in the brain, five (0.76%) miRNAs were significant, while one out of 2 (50%) was in mild AD. Meta-analysis showed a significant effect size difference in 3 (4%) different miRNAs in the temporal cortex, 2 (20%) in the hippocampus, while none of the two in the prefrontal cortex were shown to have differential expression. The subgroup analysis results of CSF specimen according to pre-mortem AD and control patients revealed 10 with a significant difference in 167 miRNAs (5.99%), whereas one out of 13 miRNAs analyzed post-mortem differed significantly (7.69%). Regarding the comparison of miRNA concentrations between AD patients and HC in blood with the PCR method, 7 out of 21 miRNAs (33.33%) were found to have a differential expression. Among 17 miRNAs in serum, 14 (82.35%) showed significant differences. Other sources such as whole blood, plasma and peripheral blood mononuclear cells (PBMC) could not be analyzed due to a lack of data. There was a risk of bias due to small number of included study in each subgroup analysis.

Correlation analysis. Fifty-seven miRNAs were reported in both brain- and CSF-derived specimens. Among them, elevated expression in both specimens was detected in 20 miRNAs and decreased expression in 7 miRNAs.

Upregulation of 9 miRNAs and downregulation of 3 miRNA was seen in 28 common miRNAs found in blood and CSF, while in both brain and blood 2 miRNA were upregulated and 4 were downregulated among 13 common miRNAs. **Supplementary Table 11** shows correlation analysis of miRNA concentrations between the 3 sources which showed no significance. However, a significant correlation was observed between brain qRT-PCR and sequencing data results. An additional correlation analysis for subgroup analyzed data was not statistically significant. SMD and Hedges' *g* were found to have a high correlation in all

sources of AD and MCI. MiRNAs that appear to have significant differential expression that play an important role in AD pathophysiology are drawn as a Venn diagram in **Supplementary Figure 1** to show the overlap between the 3 sources. One miRNA, miR-103a-3p, overlapped between all sources.

Investigation of heterogeneity. Among meta-analyses with AD, small heterogeneity was found for 291 out of 420 total meta-analyses (69.29%), moderate heterogeneity for 72 (17.14%), and high heterogeneity for 57 (13.57%). In all three specimens, a majority of the meta-analyses had low heterogeneity: 71.88% in blood, 70.10% in CSF, and 54.55% in brain.

Functional enrichment analysis results

Complete functions of all meta-analyzed miRNAs are summarized in **Supplementary Table 12**. MiRNAs that demonstrated significant differential expression in AD were involved in APP regulation, apoptosis, ubiquitin-proteasome system regulation, and neuroinflammation. **Figure 3** illustrates a schematic diagram of miRNAs and their function in the pathogenesis of AD in blood, brain, and CSF.

There were several pathways identified from functional enrichment analysis with GO enrichment analysis of 145 selected miRNAs identified several significantly enriched pathways (60 miRNAs for CSF and brain, 33 for blood). From miRNAs in all types of sources, the immune response and inflammation pathway were highly dysregulated, suggesting that these responses are the main pathogenetic pathways involved in AD. Although these pathways are highly enriched for all types of sources, especially, in blood samples, down-regulated miRNAs overlap with immune response and inflammation pathways (**Figure 4**).

DISCUSSION

The present study provides a comprehensive meta-analysis of miRNA concentrations in the brain, blood and CSF of AD, MCI patients and HC. The potential value of miRNAs as a biomarker of AD can be seen by the fact that miRNAs have a significant role in neurological diseases, the aging process, and neuroinflammation, as well as amyloid and tau pathogenesis.[24-26] Although participants with CSF examinations had the smallest proportion (10.19% of AD and 14.39% of HC), the proportion of miRNAs extracted in CSF reported in the papers was the largest (88.8%). The subgroup analysis of severe AD with blood specimen based on AD severity, in the prefrontal cortex by brain region, and ante-mortem CSF samples compared to post-mortem patients CSF samples showed a more significant effect size difference than in the meta-analyses for all patients. These results can be explained by postmortem RNA degradation, which is a major concern in research with human postmortem tissue[27]. The subgroup analysis of miRNAs found in serum was not significantly different from the value of the pooled analysis of all blood miRNAs. The correlation analysis showed no significant relationship between the 3 sources, but PCR and sequencing data revealed a significant correlation in brain specimen by the experimental methods used to identify miRNAs.

Functional analyses identified up to 129 pathways and these significant pathways were associated with 33 miRNAs in blood, 60 miRNAs in the brain, and 60 miRNAs in CSF. The immune response was the most frequently regulated pathway with 17 blood- and 34 CSF-derived miRNAs involved, while there were 38 miRNAs associated with apoptosis pathways in CSF. In the brain, the tumor suppressor function counted 24 miRNAs while the cell differentiation and cell division miRNA counted 20 and 12, respectively. Functional analyses suggest that inflammation control in AD has a critical role in prevention and treatment of the

disease.

Overall, we identified 334 miRNAs with 56 miRNAs showing significant differential expression in AD compared with cognitively normal controls (**Table 1**). Among them, 10 novel miRNAs (4 in brain, 6 in CSF) were found, which were not reported in the eight previously published meta-analyses. Two of them were from RNA sequencing data and one from data extracted from a graph. In blood, miR-103 and miR-107 (which are believed to be involved in neurite transportation and BACE1 regulation respectively) (**Supplementary table 12**) were both significantly downregulated in AD patients. However, results of miR-103 level showed high heterogeneity with $I^2 = 84.239$ while miR-107 showed zero I^2, meaning all the data were in agreement. The RNA deep sequencing data had a substantial effect in our meta-analysis since subgroup analysis with PCR of miR-132 and miR-26 showed significant differential expression while pooled results did not. MiR-132 is also upregulated in MCI, and its function is known to be related to tau phosphorylation, synaptic activity, and plasticity.[24] A number of miRNAs that have been previously reported as major factors in AD showed high heterogeneity, including miR-128, miR-125b in blood, miR-132-3p, miR-132-5p, miR-26b-5p, and miR-146a-5p in brain, and miR-125b-5p in CSF. In CSF, miR-128, miR-195 and miR-133b all showed small heterogeneity, but their p-values were not significant.

Furthermore, the PCR and sequencing data from brain source samples were revealed to be significantly associated with each other. The opposite directional regulation of the same miRNA in different parts of the body could be caused by transfer of miRNAs from one organ to another, which is supported by the fact that vesicles with biomolecules have been detected in CSF.[28] This finding of different changes in the concentration of biomolecules in various organs is consistent with previous research.[29, 30] It was also suggested that the conflicting change of miRNA concentrations between tissues and plasma may be due to the cellular

selection mechanism of releasing miRNA.[31]

We also searched for lncRNA, which has drawn special attention in cancer research,[32] but there were very few reports on lncRNA that could be included in a meta-analysis. The expression of the only lncRNA eligible for meta-analysis, BACE1-AS, was found to be nonsignificant in the meta-analysis. Further research on the expression of lncRNA in AD is needed.

As shown in **Figure 2**, miRNAs that inhibit multiple pathways leading to AD pathogenesis such as miR-15a-5p, miR-103a-3p, and miR-29a-3p could be valuable therapeutic candidates for the treatment of AD. Pathway analysis showed that the mechanisms that are associated with Aβ clearance are highly dysregulated, including autophagic processes, inflammation, and toxicity (**Figure 4**). The considerably disrupted regulation of lipid metabolism, adipogenesis and adipocyte differentiation found in blood and brain is consistent with previous reports of decreased lecithin cholesterol acyl transferase (LCAT) activity, membrane destabilization and the association of ApoE with Aβ in AD patients (**Figure 4**).[33-35] The reason for the elevated functional enrichment results of onco-miRNAs and tumor suppressor miRNAs is that a number of miRNAs play a crucial role in apoptosis, DNA damage, cellular stresses and cell cycle regulation as shown in **Figure 2**. Apoptosis appears to be crucial in AD as pro-apoptotic molecules increase and neuroprotective pathways are initiated.[36, 37]

Our study elucidated numerous functional enrichment pathways associated with AD. Cholinergic, Aβ formation, tau hyperphosphorylation and inflammation pathways play a crucial role in the pathogenesis of AD as suggested previously[38]. It has been suggested previously that, Aβ senile plaques appear during the microglial activation stage and are responsible for inducing the oxidative stage, promoting tau hyperphosphorylation[39, 40]. It is

particularly noteworthy that miRNAs with significant meta-analysis results were highly dysregulated in the immune response pathway. To interfere with neuroinflammation in the disease process, several studies used anti-inflammatory drugs such as COX inhibitors and non-steroidal anti-inflammatory drugs[41, 42]. Nowadays, there are several clinical trials ongoing regarding monoclonal antibody targeting amyloid plaques, however, we suggest targeting more specifically on the immunologically active site of amyloid plaques.

While there has been no meta-analysis with SMD in AD reported so far, we searched and scrutinized more recent papers in detail. Hu et al. evaluated and meta-analyzed 7 articles, but they did not demonstrate the analysis results with SMD and CI.[13] Takousis et al. applied Stouffer's method which uses *P* values rather than differences in the expressed miRNA concentrations.[18] The most recent meta-analysis in this area of research screened 895 articles and meta-analyzed 295 miRNAs while we screened 2,024 articles and meta-analyzed 328 miRNAs in total (**Table 1**).[18] While meta-analyses of miRNA regulation in AD have already been published, our work included more recent articles with specific concentrations of each miRNA. Also, we extracted approximately 46% of datasets from figures. The insufficient reporting of results, in a large proportion of studies included in the meta-analysis, may lead to bias and quality issues, impacting the overall findings. Thus, a subgroup analysis was done to judge how robust the overall findings are. We additionally conducted a functional enrichment study to provide further in-depth investigations in this crucial field of AD pathogenesis. However, some sources were not able to be analyzed due to lack of data. Especially, the consideration of blood as a single source could obscure less abundant miRNA such as serum and plasma with highly abundant miRNA such as PBMCs.

Although we report differential expression of newly detected miRNAs in AD, our study has several potential limitations. First, we may not have included all articles published to date

due to limitations in the literature search. Even if we have included all eligible papers, there was a lack of raw data in some of them. Second, relatively large heterogeneity was observed among the studies. Information on the study group's sex, age, ethnicity, were not always provided, and there were some differences in the diagnostic criteria. Also, there was a large imbalance between the number of AD and HC participants used in the brain meta-analysis. Third, due to methodological variation relating to PCR, the cycle threshold (CT) value was not consistently presented, as there is no consensus on how the CT value should be normalized. To overcome this problem, we used Hedges' *g*, which uses pooled weighted standard deviations. Hedges' g measures effect size, which is an index that can be used regardless of the unit of individual data. Also, a variety of housekeeping genes were used in the qPCR-based studies. Among them, U6 snRNA and RNU43 were the most used. However, this variance in housekeeping genes could cause confounding errors in the analysis.

Fifth, the mean NOS of the included studies was 5.57, which is not considered high. Studies with a low NOS score might have skewed the analysis outcome. In addition to the unit of the PCR results and NOS value, different experimental methods, and differences in housekeeping genes could have also introduced bias in the analysis across datasets. Nevertheless, by including patients at the two ends of the AD spectrum, heterogeneity could have been reduced. Sixth, we searched two database according to the PRISMA guideline.[20] However, it has been reported that searching only one database is sufficient for systematic reviews and meta-analyses,[43] and that searching Pubmed only has a 10% risk of the primary outcome odds ratio changing by >20%.[44] Lastly, the regulations of miRNAs from different parts of AD patients are different (up-regulated or down-regulated) and probably represent different pathophysiologies. So, pooled-analysis of previously reported different miRNAs with functional enrichments to reveal dysregulated processes in AD should be interpreted

with caution.

In conclusion, our meta-analysis and systematic review demonstrated that 425 miRNAs among 61 individual studies were differentially expressed between AD patients and HC in blood, brain and CSF. Despite intensive research, miRNA and their role in AD is still in its infancy, because until now, there has been a lack of methodological consistency in previous studies. An implication of these findings is that miRNAs have potential as diagnostic and therapeutic targets. Because AD remains a major health concern in elderly people worldwide, further studies on the role of miRNA in AD pathogenesis are needed.

ACKNOWLEDGEMENTS AND DISCLOSURES

This paper demonstrates independent research and all authors are acknowledged for their contributions to this study.

Author Contributions

SY and JIS designed the study. SY, SUK and JIS searched the literature, and extracted data. Any discrepancies were resolved via discussion between SY, SUK and JIS. SY, SUK, YK, GHJ, KHL and JIS undertook the statistical analyses and interpreted the data. SY and SUK made the figures and tables. All authors drafted and critically revised the manuscript. All authors approved the final version of the manuscript for publication.

Conflict of interest

None reported.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Figure legends

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart.

Abbreviations: MiRNA, microRNA; ncRNA, non-coding RNA; AD, Alzheimer's disease; SD, standard deviation

Figure 2. Forest plot for random-effects meta-analysis and important functions of miRNAs.

(a) Meta-analysis results of patients with Alzheimer's disease in each specimen.

(b) Meta-analysis results of patients with mild cognitive impairment in blood specimen.

Abbreviations: MiRNA, microRNA; CI, 95% confidence interval; LCI, lower 95% confidence interval; UCI, upper 95% confidence interval; CSF, cerebrospinal fluid

Figure 3. Schematic diagram of miRNA and their pathogenesis in Alzheimer's disease

(a) MiRNAs detected in the blood. The figure summarizes the Aβ and tau hypothesis of AD pathogenesis. The UPS pathway and microglia are involved in Aβ clearance and neuroinflammation. BACE1-AS is a long noncoding RNA (lncRNA).

(b) MiRNAs detected in the brain. Top, the aggregation of Aβ peptide leads to the formation of amyloid plaques. Bottom left, tau hyperphosphorylation results in toxic neurofibrillary tangles. Bottom right, miRNAs regulate apoptosis and autophagy, thus bringing neuroinflammation and neurodegeneration.

(c) MiRNAs detected in the CSF. Neuronal dysfunction and death, neuroinflammation is associated with AD.

Abbreviations: ADAM10, A disintegrin and metalloproteinase 10; APP, amyloid precursor protein; BACE1, beta-secretase 1; Aβ, amyloid beta; ABCA1, ATP binding cassette transporter A1; ApoE, apolipoprotein E; UPS, ubiquitin-proteasome system; UBE2N, ubiquitin-conjugating enzyme E2 N; USP2, ubiquitin-specific protease 2; BAP1, BRCA1-associated protein 1; BBB, blood-brain barrier; LRP, low density lipoprotein (LDL) receptor-related protein; LRPAP1, LRP associated protein 1; MMP, matrix metalloproteinase ; CBX4, chromobox 4; SMURF1, SMAD ubiquitylation regulatory factor 1; SR-B1, scavenger receptor, class B type 1; ERK, extracellular signal-regulated kinase; TLR, toll like receptor; SIRT1, sirtuin 1; MAPK, mitogen-activated protein kinase; GSK, glycogen synthase kinase; AKT, protein kinase B; PI3K, phosphatidylinositol 3-kinase; IKK, IκB kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PPAR-γ, peroxisome proliferator-activated receptor gamma; mTORC1, mammalian target of rapamycin complex 1; RAPTOR, regulatory-associated protein of mTOR; ULK, unc-51 like autophagy activating kinase; ATG, beclin-1-associated autophagy-related key regulator; BECN1, human beclin 1 gene; SPT, serine palmitoyltransferase; STAT5, signal transducer and activator of transcription 5; JAK2, janus kinase 2; RAS, rat sarcoma; ERK, extracellular signal-regulated kinase; Nrf2, nuclear factor erythroid 2-related factor 2; SOD2, superoxide dismutase 2; BDNF, brain-derived neurotrophic factor; VPS34, vacuolar protein sorting 34; CSF, cerebrospinal fluid

Figure 4. Functional enrichment analysis results

The top 60 miRNAs from blood were selected and the enriched functional pathways (p-value < 0.05) associated with these miRNAs were identified. Abbreviations: MiRNA, microRNA

(a) Blood-derived miRNA.

(b) Brain-derived miRNAs.

(c) CSF-derived miRNAs.

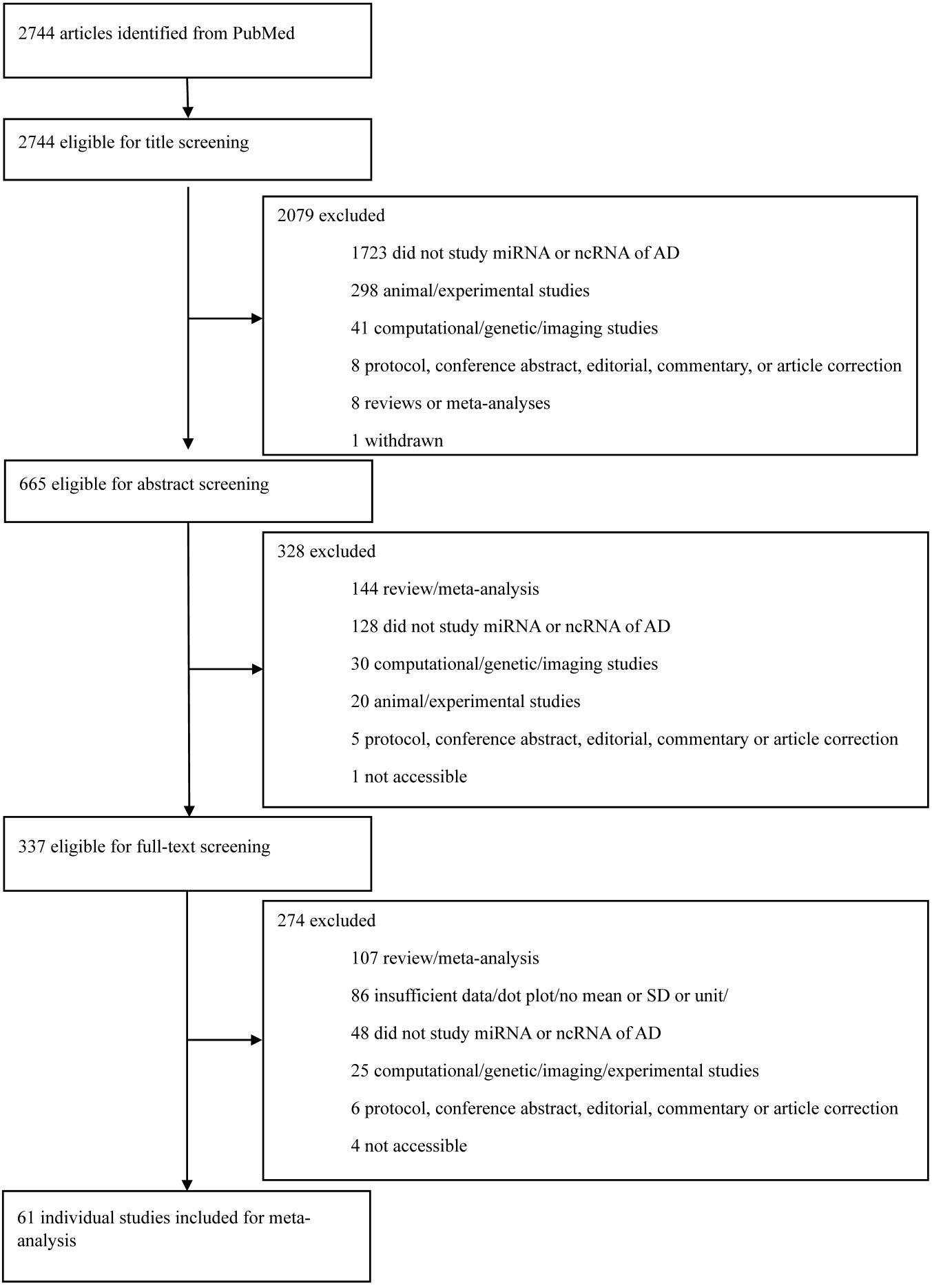
Abbreviations: MiRNA, microRNA; CSF, cerebrospinal fluid

Table 1. Main results of the meta-analysis.

	Number						
Articles screened	2744						
Articles included	61						
Total participants	6,853 (AD: 3,442; MCI: 302; HC: 3,109)						
Total data	3,560 (AD: 3,534; MCI: 26)						
Meta-analyzed data	1,187						
Total miRNAs	1512						
Meta-analyzed miRNAs	425						
	Number of participants		**Number of data**	**Meta-analyzed data**	**Number of miRNA**	**Meta-analyzed miRNA**	**Significant results[a]**
	Patients	**HC**					
Blood	2,787	2,726	162	82	111	33	19 (57.58%)
Brain	741	436	836	244	689	96	8 (8.33%)
CSF	379	353	2,536	851	1,238	291	29 (9.97%)
MCI	302	408	26	10	21	5	5 (100.00%)
Significant results	**MiRNAs[b, c]**						
Blood	hsa-miR-30e-5p, **hsa-miR-18b-5p, hsa-miR-424-5p, hsa-miR-582-5p, hsa-miR-335-5p,** hsa-miR-20a-5p, **hsa-miR-106a-5p, hsa-miR-361-5p,** hsa-miR-15a-5p, hsa-miR-29b-3p, hsa-miR-27b-3p, hsa-miR-221-3p, hsa-miR-146a-5p, hsa-miR-15b-3p, hsa-miR-31-5p, hsa-miR-9-5p, hsa-miR-107, hsa-miR-103a-3p, **hsa-miR-1306-5p**						
Brain	hsa-miR-455-3p, ***bta-miR-2476, hsa-mir-4776-2, hsa-miR-4315,*** hsa-miR-195-5p, hsa-miR-107, hsa-miR-103a-3p, ***oan-miR-1386***						
CSF	***hsa-miR-629-5p,*** **hsa-miR-539-5p,** ***hsa-miR-1264,*** **hsa-miR-501-5p, hsa-miR-214-5p, hsa-miR-381-3p,** ***hsa-miR-643,*** hsa-miR-34c-3p, **hsa-miR-20b-5p,** hsa-miR-144-3p, hsa-455-3p**, hsa-miR-127-3p** ***hsa-miR-1911-5p, hsa-miR-636,*** **hsa-miR-497-5p, hsa-miR-511-3p, hsa-miR-181d-5p,** hsa-miR-15a-5p**, hsa-miR-106a-5p,** hsa-miR-29a-3p, **hsa-miR-30a-3p,** hsa-let-7i-5p, **hsa-miR-31-5p,** hsa-miR-103a-3p**, hsa-miR-181a-5p,** hsa-miR-362-3p, **hsa-miR-320b,** hsa-miR-146a-5p, *hsa-miR-605-5p*						
MCI	hsa-miR-874-3p, hsa-miR-132-3p, hsa-miR-128-3p, hsa-miR-323a-3p, hsa-miR-134-3p						

[a] Significant meta-analysis results from random effects model.

[b]MiRNAs not reported in previous meta-analyses are in italics. [c]MiRNAs not listed in the abstract of included original studies are in bold.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41 computational/genetic/imaging studies
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8 reviews or meta-analyses
1 withdrawn
665 eligible for abstract screening
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144 review/meta-analysis
128 did not study miRNA or ncRNA of AD
30 computational/genetic/imaging studies
20 animal/experimental studies
5 protocol, conference abstract, editorial, commentary or article correction
1 not accessible
337 eligible for full-text screening
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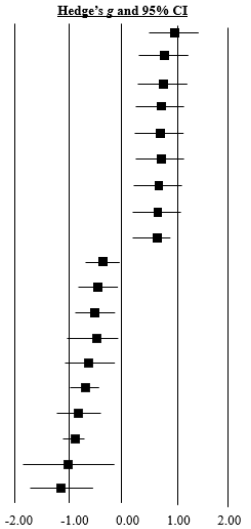
107 review/meta-analysis
86 insufficient data/dot plot/no mean or SD or unit/
48 did not study miRNA or ncRNA of AD
25 computational/genetic/imaging/experimental studies
6 protocol, conference abstract, editorial, commentary or article correction
4 not accessible
61 individual studies included for meta-analysis

(a)

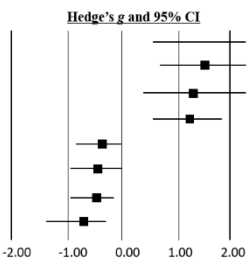
Blood

MiRNA	Function	Hedges's g	LCI	UCI	p-value
hsa-miR-30e-5p		0.903	0.478	1.328	0.000
hsa-miR-18b-5p		0.776	0.355	1.197	0.000
hsa-miR-424-5p		0.761	0.341	1.182	0.000
hsa-miR-582-5p		0.676	0.259	1.094	0.002
hsa-miR-335-5p		0.665	0.248	1.081	0.002
hsa-miR-20a-5p		0.654	0.211	1.097	0.004
hsa-miR-106a-5p		0.608	0.192	1.024	0.004
hsa-miR-361-5p		0.607	0.192	1.022	0.004
hsa-miR-15a-5p		0.547	0.152	0.941	0.007
hsa-miR-29b-3p		-0.344	-0.665	-0.023	0.036
hsa-miR-27b-3p		-0.442	-0.813	-0.071	0.020
hsa-miR-221-3p		-0.496	-0.868	-0.123	0.009
hsa-miR-146a-5p		-0.534	-1.014	-0.055	0.029
hsa-miR-15b-3p		-0.668	-1.101	-0.235	0.002
hsa-miR-31-5p		-0.728	-0.983	-0.474	0.000
hsa-miR-9-5p		-0.792	-1.206	-0.378	0.000
hsa-miR-107		-0.897	-1.099	-0.695	0.000
hsa-miR-103a-3p		-1.083	-1.960	-0.206	0.016
hsa-miR-1306-5p		-1.147	-1.803	-0.491	0.001



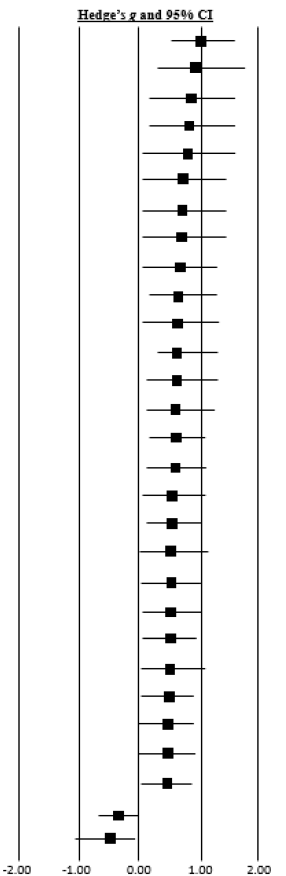
Brain

MiRNA	Function	Hedges's g	LCI	UCI	p-value
hsa-miR-455-3p		3.165	0.574	5.756	0.000
hsa-mir-4776-2		1.409	0.664	2.155	0.000
bta-miR-2476		1.267	0.318	2.216	0.009
hsa-miR-4315		1.170	0.444	1.896	0.002
hsa-miR-195-5p		-0.474	-0.899	-0.05	0.028
hsa-miR-107		-0.503	-0.917	-0.089	0.017
hsa-miR-103a-3p		-0.535	-0.96	-0.11	0.014
oan-miR-1386		-0.875	-1.401	-0.35	0.001



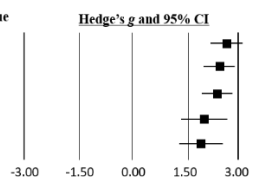
CSF

MiRNA	Function	Hedges's g	LCI	UCI	p-value
hsa-miR-629-5p		1.039	0.504	1.574	0.000
hsa-miR-539-5p		0.934	0.225	1.642	0.010
hsa-miR-1264		0.884	0.153	1.615	0.018
hsa-miR-501-5p		0.832	0.106	1.558	0.025
hsa-miR-214-5p		0.818	0.037	1.600	0.040
hsa-miR-381-3p		0.775	0.056	1.495	0.035
hsa-miR-643		0.772	0.048	1.496	0.037
hsa-miR-34c-3p		0.751	0.065	1.438	0.032
hsa-miR-20b-5p		0.716	0.198	1.234	0.007
hsa-miR-144-3p		0.710	0.108	1.312	0.021
hsa-miR-455-3p		0.706	0.068	1.343	0.030
hsa-miR-127-3p		0.697	0.222	1.172	0.004
hsa-miR-1911-5p		0.660	0.111	1.209	0.019
hsa-miR-511-3p		0.649	0.134	1.164	0.014
hsa-miR-636		0.646	0.204	1.088	0.004
hsa-miR-497-5p		0.639	0.197	1.081	0.005
hsa-miR-181d-5p		0.593	0.080	1.105	0.023
hsa-miR-106a-5p		0.583	0.182	0.985	0.004
hsa-miR-15a-5p		0.571	0.005	1.137	0.048
hsa-miR-29a-3p		0.570	0.052	1.089	0.031
hsa-miR-30a-3p		0.561	0.120	1.001	0.013
hsa-let-7i-5p		0.549	0.150	0.949	0.007
hsa-miR-31-5p		0.536	0.053	1.019	0.030
hsa-miR-103a-3p		0.463	0.065	0.860	0.022
hsa-miR-181a-5p		0.440	0.043	0.837	0.030
hsa-miR-362-3p		0.440	0.004	0.877	0.048
hsa-miR-320b		0.420	0.024	0.817	0.038
hsa-miR-146a-5p		-0.325	-0.645	-0.005	0.046
hsa-miR-605-5p		-0.587	-1.120	-0.053	0.031

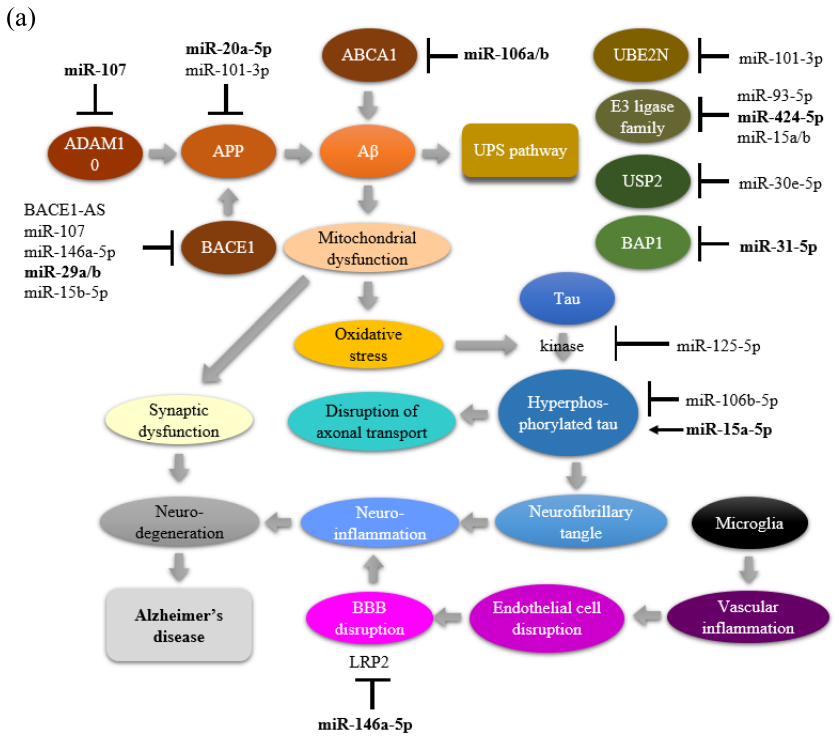


(b)

MiRNA	Function	Hedges's g	LCI	UCI	p-value
hsa-miR-874-3p		2.651	2.200	3.103	0.000
hsa-miR-132-3p		2.457	2.020	2.893	0.000
hsa-miR-128-3p		2.387	1.956	2.818	0.000
hsa-miR-323a-3p		2.024	1.376	2.672	0.000
hsa-miR-134-3p		1.935	1.324	2.547	0.000



Amyloid related function
Tau related function
Apoptosis & cell proliferation function
Immune response & inflammation
Other function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UBE2N
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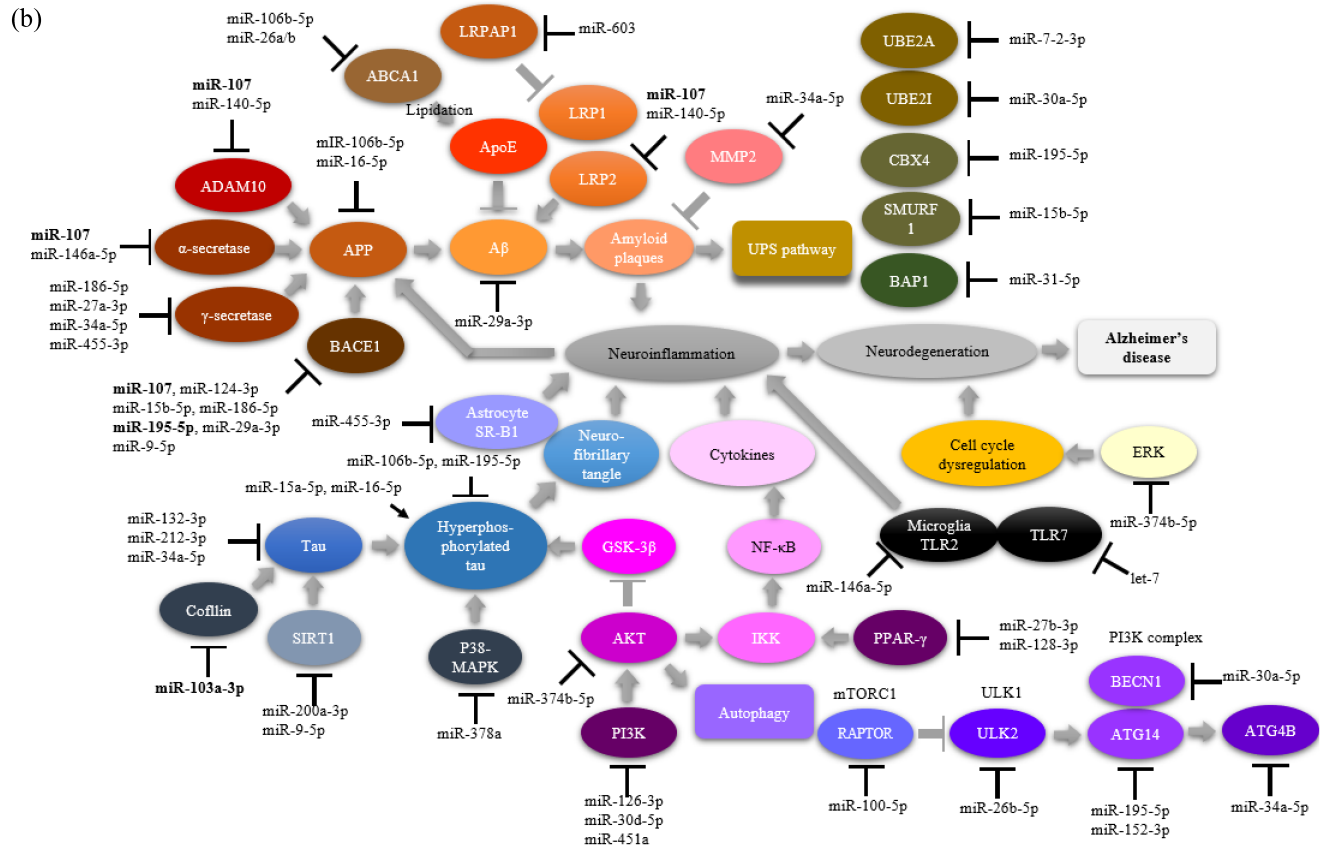
miR-93-5p
miR-424-5p
miR-15a/b
USP2
miR-30e-5p
BAP1
miR-31-5p
ADAM10
APP
Aβ
UPS pathway
BACE1-AS
miR-107
miR-146a-5p
miR-29a/b
miR-15b-5p
BACE1
Mitochondrial dysfunction
Oxidative stress
Tau
kinase
miR-125-5p
Synaptic dysfunction
Disruption of axonal transport
Hyperphos-phorylated tau
miR-106b-5p
miR-15a-5p
Neuro-degeneration
Neuro-inflammation
Neurofibrillary tangle
Microglia
Alzheimer's disease
BBB disruption
Endothelial cell disruption
Vascular inflammation
LRP2
miR-146a-5p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Lipidation
LRP1
miR-107
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ApoE
LRP2
MMP2
miR-107
miR-140-5p
ADAM10
mIR-106b-5p
miR-16-5p
APP
Aβ
Amyloid plaques
UPS pathway
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γ-secretase
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miR-27a-3p
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Neurodegeneration
Alzheimer's disease
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miR-9-5p
miR-455-3p
Astrocyte SR-B1
Neuro-fibrillary tangle
Cytokines
miR-106b-5p, miR-195-5p
Cell cycle dysregulation
ERK
miR-374b-5p
miR-15a-5p, miR-16-5p
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miR-34a-5p
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Hyperphos-phorylated tau
GSK-3β
NF-κB
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TLR7
miR-146a-5p
let-7
Cofilin
SIRT1
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miR-9-5p
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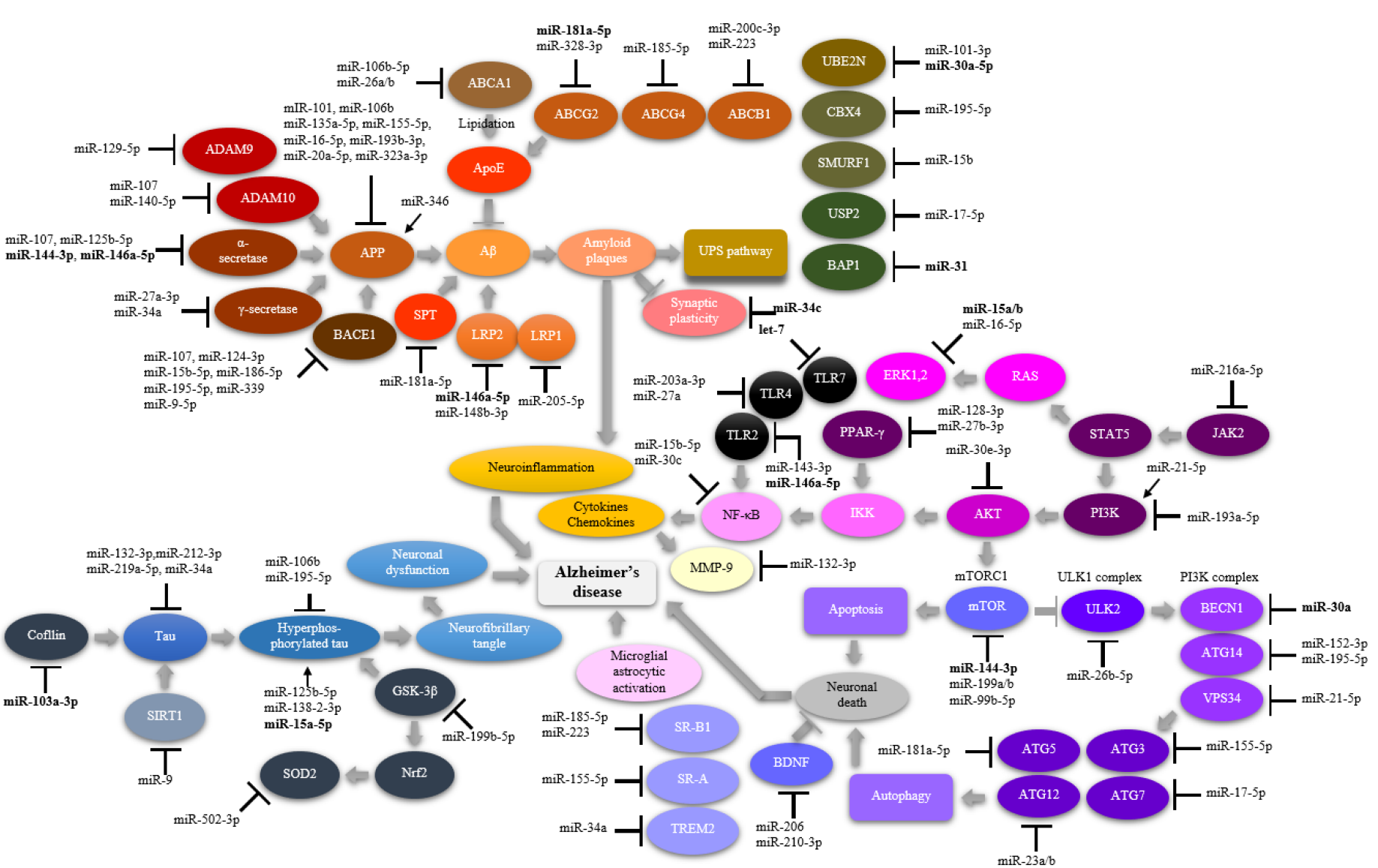
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Lipidation
ApoE
miR-129-5p
ADAM9
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α-secretase
APP
Aβ
Amyloid plaques
UPS pathway
miR-27a-3p
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BACE1
SPT
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miR-195-5p, miR-339
miR-9-5p
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miR-148b-3p
miR-205-5p
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miR-34c
let-7
miR-15a/b
miR-16-5p
miR-203a-3p
miR-27a
TLR4
TLR7
ERK1,2
RAS
miR-216a-5p
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STAT5
JAK2
miR-15b-5p
miR-30c
miR-143-3p
miR-146a-5p
miR-30e-3p
miR-21-5p
Neuroinflammation
Cytokines
Chemokines
NF-κB
IKK
AKT
PI3K
miR-193a-5p
MMP-9
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miR-219a-5p, miR-34a
miR-106b
miR-195-5p
Neuronal dysfunction
Alzheimer's disease
mTORC1
ULK1 complex
PI3K complex
Apoptosis
mTOR
ULK2
BECN1
miR-30a
Cofilin
Tau
Hyperphos-phorylated tau
Neurofibrillary tangle
miR-144-3p
miR-199a/b
miR-99b-5p
miR-26b-5p
ATG14
miR-152-3p
miR-195-5p
miR-103a-3p
Microglial astrocytic activation
Neuronal death
VPS34
miR-21-5p
SIRT1
miR-125b-5p
miR-138-2-3p
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GSK-3β
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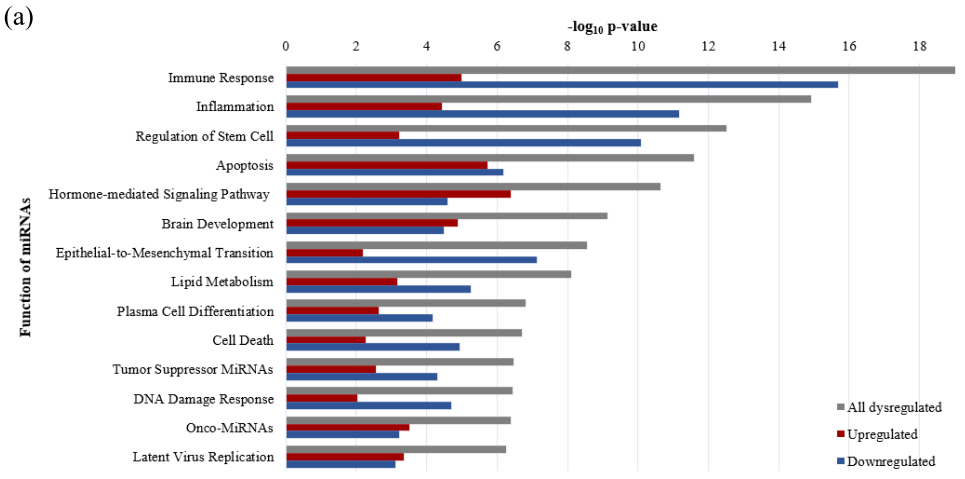
Nrf2
miR-502-3p
miR-185-5p
miR-223
SR-B1
miR-155-5p
SR-A
miR-34a
TREM2
BDNF
miR-206
miR-210-3p
miR-181a-5p
ATG5
ATG3
miR-155-5p
Autophagy
ATG12
ATG7
miR-17-5p
miR-23a/b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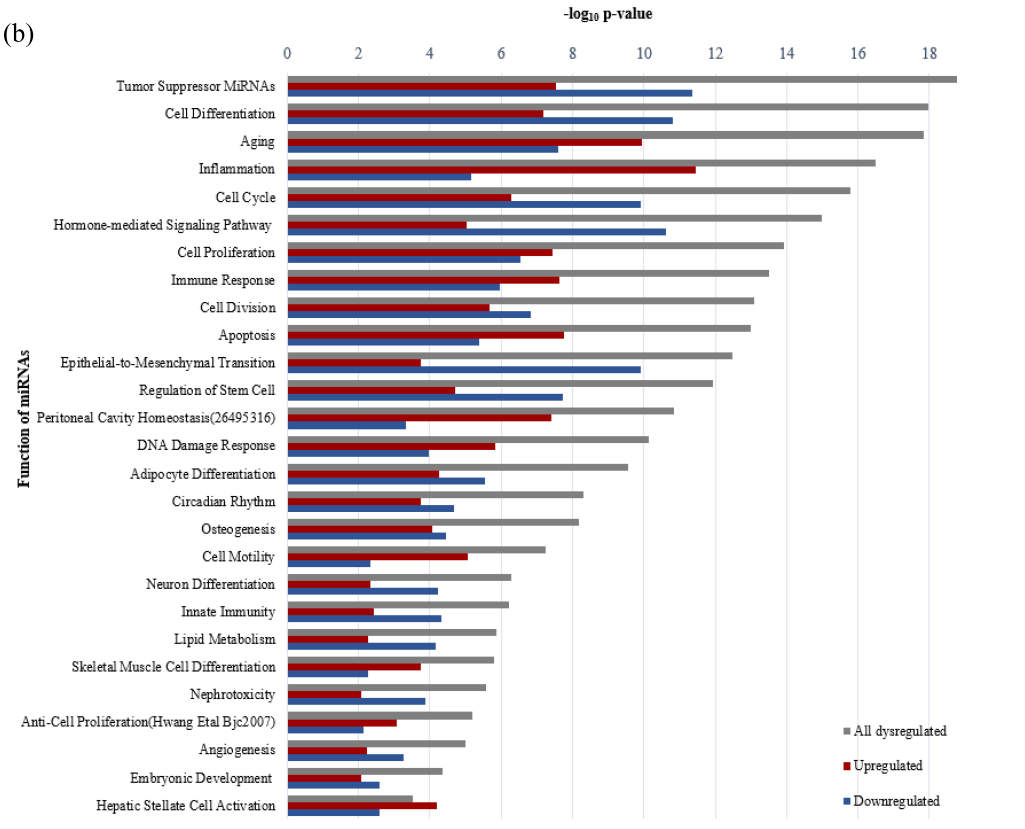
Function of miRNAs
Immune Response
Inflammation
Regulation of Stem Cell
Apoptosis
Hormone-mediated Signaling Pathway
Brain Development
Epithelial-to-Mesenchymal Transition
Lipid Metabolism
Plasma Cell Differentiation
Cell Death
Tumor Suppressor MiRNAs
DNA Damage Response
Onco-MiRNAs
Latent Virus Replication
All dysregulated
Upregulated
Downregulated


(b)
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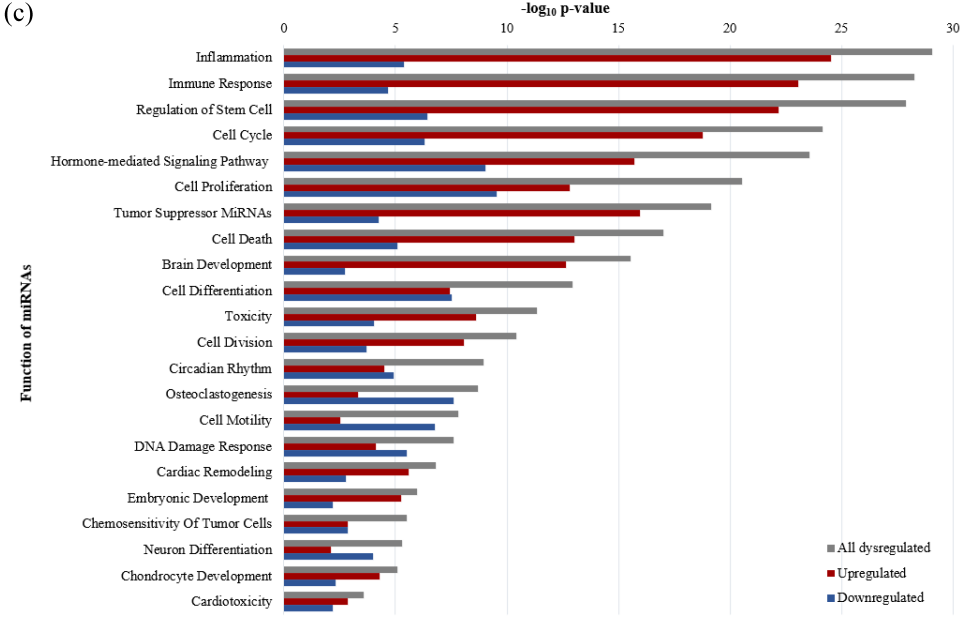
Function of miRNAs
Tumor Suppressor MiRNAs
Cell Differentiation
Aging
Inflammation
Cell Cycle
Hormone-mediated Signaling Pathway
Cell Proliferation
Immune Response
Cell Division
Apoptosis
Epithelial-to-Mesenchymal Transition
Regulation of Stem Cell
Peritoneal Cavity Homeostasis(26495316)
DNA Damage Response
Adipocyte Differentiation
Circadian Rhythm
Osteogenesis
Cell Motility
Neuron Differentiation
Innate Immunity
Lipid Metabolism
Skeletal Muscle Cell Differentiation
Nephrotoxicity
Anti-Cell Proliferation(Hwang Etal Bjc2007)
Angiogenesis
Embryonic Development
Hepatic Stellate Cell Activation
All dysregulated
Upregulated
Downregulated


(c)
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Function of miRNAs
Inflammation
Immune Response
Regulation of Stem Cell
Cell Cycle
Hormone-mediated Signaling Pathway
Cell Proliferation
Tumor Suppressor MiRNAs
Cell Death
Brain Development
Cell Differentiation
Toxicity
Cell Division
Circadian Rhythm
Osteoclastogenesis
Cell Motility
DNA Damage Response
Cardiac Remodeling
Embryonic Development
Chemosensitivity Of Tumor Cells
Neuron Differentiation
Chondrocyte Development
Cardiotoxicity
All dysregulated
Upregulated
Downregulated