# The role of microRNAs to understand sex-based differences in Alzheimer's disease

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## Abstract

**Background:** Alzheimer's disease (AD) is the most frequent cause of dementia and its incidence is expected to rise as the life expectancy of the population increases. Sex-based differences in AD development have been described, although there are still uncertainties on the role of biological sex in molecular mechanisms of the disease. The study of sex-specific expression profiles of regulatory elements, such as microRNAs (miRNAs), could contribute to a more accurate diagnosis and treatment of the disease.

**Methods:** We conducted a systematic review identifying five studies of microRNA expression in AD that incorporated the biological sex of the samples published in the Gene Expression Omnibus (GEO) repository. Differential expression analyses were performed for each study, considering both disease and biological sex of patients. Subsequently, results were integrated with a meta-analysis methodology. Finally, functional enrichment of the meta-analyses results was performed to establish an association between altered miRNA expression and relevant terms of the Gene Ontology (GO).

**Results:** Meta-analyses of miRNAs expression in blood samples revealed 16 and 22 miRNAs altered in females and males, respectively. Moreover, 9 miRNAs were commonly overexpressed in both sexes, unveiling common miRNAs dysregulation profiles. In contrast, functional enrichment revealed sex-differences in biological processes altered. In males, most of the affected processes were related to ubiquitination, regulation of different kinase activities and apoptotic processes; but in females were linked to RNA splicing and translation. Meta-analyses of brain samples also revealed some alterations in miRNAs expression (6 in females and 4 in males). The unique miRNA altered in both sexes (miR-767-5p) was underexpressed in both males and females. Nonetheless, the functional enrichment analysis did not reveal any affected biological process.

**Conclusions:** Sex-specific meta-analyses allowed for the detection of differentially expressed miRNAs in females and males. Identification of deregulated miRNAs highlighted the relevance of the sex information in biomedical data. Further studies centered on miRNA regulation should meet criteria

for comparability and standardization of information.

**Keywords:** sex-differences, Alzheimer, systematic review, meta-analysis, transcriptomics, microRNA

**Highlights:**

- miRNA expression profile is deregulated in AD in a tissue- and sex-specific manner.
- Meta-analysis of blood and brain samples revealed a partial overlapping of altered miRNAs expression in males and females AD patients.
- Functional enrichment of AD altered miRNAs expression in blood samples reveal sex-based differences: RNA splicing and translation in females and ubiquitination, regulation of different kinase activities and apoptotic process in males.

**Plain English Summary:**

Alzheimer's disease (AD) is a neurodegenerative disease that affects mostly aged people. AD symptomatology is characterized by cognitive deterioration, memory loss and progressive incapacitation in daily activities. Considering sex differences, AD affects almost twice as many females than males. Moreover, cognitive deterioration and brain atrophy are faster in females. Nevertheless, the biological causes of these differences are poorly understood. miRNAs are small fragments of RNA that regulate gene expression in many biological processes. Therefore, the study of miRNAs in females and males with AD could contribute to a better understanding of the disease. Thus, we reviewed all studies about miRNA expression in females and males with AD and integrated their results with a meta-analysis methodology. Finally, we looked for those genes regulated by affected miRNAs to establish an association with biological processes. We found 16 and 22 miRNAs altered the blood of females and males, respectively. Moreover, functional enrichment revealed sex-differences in biological processes altered. In males AD, most of the affected processes were related to RNA processing, whereas in females AD, altered processes were linked to protein modification

and degradation, as well as cell death. In the brain, we found 6 miRNAs altered in females and 4 miRNAs in males. Nonetheless, our analysis did not find biological processes altered in the brain of AD patients. To sum up, we found differentially expressed miRNAs in females and males, mainly in the blood but also in the brain. Moreover, the biological processes affected in the blood were different in females and males.

## Background

Alzheimer's disease (AD) is the most common progressive neurodegenerative disease that causes dementia in elderly population, although approximately 5%-10% of all AD cases start to develop in people under 65 (early onset AD, EOAD) [1]. The incidence of AD is estimated to triplicate by 2050 [2–4], representing a global challenge for public health systems. Its symptomatology is characterized by cognitive deterioration, memory loss and social skills that culminates in total dependency and death [5]. The main risk factors for AD are older age and having a family history of AD, the latter mainly related with EOAD and described mutations on the amyloid precursor protein (APP), presenilin-1 (PSEN1) and presenilin-2 (PSEN2) genes [6]. Regarding the late onset AD (LOAD), risk inducing mutations were identified through GWAS and candidate genes involved in several pathways: cholesterol and lipid metabolism, genes related to inflammation and immune system and endosome cycling genes [7]. Pathological hallmarks of AD include the amyloid plaques composed by aggregated β-amyloid peptide (Aβ) as a consequence of Aβ overproduction or insufficient removal, and the hyperphosphorylated tau neurofibrillary tangles extending across brain regions as the disease progresses [8–10]. Aβ accumulation leads to excitotoxicity, inflammation and oxidative stress [8] produced by microglial cells overactivation that contributes to synaptic impairment [8–10]. While hyperphosphorylated tau affects the stability of the cytoskeleton, altering both trafficking in the postsynaptic receptors and axonal transport. Besides, multiple neurotransmission systems are impaired due to the inflammatory events, including the cholinergic system, serotonergic and glutamatergic neurons. These effects, in turn, produce alterations in memory, neuroplasticity and

excitotoxicity events [9,11].

Regarding the sex differences in AD, different reports described an increased prevalence and incidence in females [12], which could be partially explained by increased life expectancy in females, as well as socio-economic factors [13]. Besides, a faster cognitive decline and atrophic rate in females is well described [14–17]. Nevertheless, the sex-based molecular profiles behind these differences and sex-specific AD biomarkers are barely explored. Currently, the diagnostic techniques for AD including cerebrospinal fluid (CSF) biomarkers and neuroimaging analysis have limited clinical application, due to their invasive nature or high cost [18,19], highlighting the need of novel biomarkers that allow more affordable and noninvasive techniques. In this sense, miRNAs in blood and sex -related represent a promising noninvasive tool that could facilitate diagnosis and tailored interventions in females and males for different reasons: I) MiRNA are small non-coding RNA molecules that regulate the expression of other genes at post-transcriptional levels. II) Their expression is conserved, temporal and tissue specific. III) MiRNAs importantly influence onset and AD pathology as they play a role in AB metabolism, Tau function, immuno-inflammatory responses and other neural events. IV) In neurological diseases and specifically in AD, miRNA differential expression based on the sex of the patients has previously been described. V) MiRNAs may be regulated by sex hormones and display a higher density in chromosome X [20][21][20][22][20][23,24][25–27]). Therefore, evaluating sex-specific miRNA patterns comprise an opportunity to improve clinical outcomes in AD by sex.

To address this, we conducted a systematic review and meta-analysis of miRNA expression studies in brain and blood of AD patients. A range of previous studies have integrated data from numerous sources to characterize sex-based differences in the human transcriptome [28–32]; however, to our knowledge, this study represents the first meta-analysis of microRNA studies in AD, which provides a better understanding of the sex-related molecular mechanisms underlying AD. We found consensus miRNAs altered in AD in both tissues. Besides, we found specific miRNAs expression signatures for males and females, especially in blood samples, unveiling novel sex-based potential

biomarkers for this disease. Finally, we functionally characterized the effects of these miRNAs alterations, describing sex-specific biological processes altered in blood samples of AD patients.

## Methods

### Study selection via systematic review

A systematic review following the PRISMA guidelines [33] was performed, searching for studies of the microRNA expression on human AD patients on the GEO and ArrayExpress databases [34,35]. The keyword selected for the search was “Alzheimer’s disease” and the results were filtered by: i) dataset type: "non coding RNA profiling by array" or "non coding RNA profiling by high throughput sequencing", and ii) organism: “Homo sapiens”.

We applied several exclusion criteria for the studies found: studies not related to AD, studies without the sex information of each patient, experimental designs different from AD patients against controls, studies not centered in miRNAs, studies on organisms other than human and studies for which expression data was not accessible.

### Bioinformatic workflow

The approach to the expression data analysis consisted on a pipeline for every study selected: i) data acquisition, ii) normalization and preprocessing (including establishing a common annotation for the miRNAs on miRBase v22 [36]) , iii) individual exploratory analysis, iv) differential expression analysis of each study, v) integration of the differential expression results with a meta-analysis for each sex and tissue, and vi) functional enrichment of the results obtained in the meta-analyses (Figure 2). All analyses carried out were performed using the R language 4.1.2 [37] and the packages required to carry out the analyses are described in Supplementay Materials.

**Data acquisition, normalization and preprocessing**

The normalized expression matrix and the sample information of each selected study was downloaded from the GEO database. Sample names and tags for the sex and health condition of each sample were homogenized to facilitate further analyses and contrasts. In studies that contained experimental groups other than control or AD, only such samples were selected.

With regards to the identifiers selected for miRNAs, all the features were annotated with miRBase v22 IDs [36] and only mature miRNAs were kept. For repeated miRNAs, the highest expression value was preserved. NA features were removed. The minimum expression value was added to the matrices to eliminate negative values, and log2 was applied to the expression values when the transformation was not done beforehand.

**Exploratory analysis of individual studies**

To get an overview and to identify potential anomalies on the data of each study, we explored the data with several representations: the proportion of patients by condition and sex, and the expression data distribution with boxplots. Furthermore, the potential categorical aggregations of the samples associated with the experimental conditions were assessed via hierarchical clustering and Principal Component Analysis (PCA).

**Differential expression analysis**

To carry out miRNAs differential expression (DE) analyses, we applied a linear model, including two contrasts to assess the effects of AD on females and males: i) (AD female - Control female) and ii) (AD male - Control male) respectively. The analysis was carried out with the limma package [28] for R. A transformation from discrete to continuous data was made for RNA-Seq studies using the “voom” function of the limma package, thus allowing the following linear model construction. According to the linear model, we were able to determine for each miRNA analyzed and each contrast, differences on the expression levels under the studied conditions. The Benjamini & Hochberg (BH) method [38] was applied to adjust the p-values for multiple comparisons.

**Meta-analysis**

The integration of the differential expression analysis for individual studies was conducted with a meta-analysis approach, grouping the studies by tissue (brain and blood). For each group of studies, the meta-analysis was applied on the results of previously proposed contrasts. Based on the considered factors, we conducted four meta-analyses (brain female, brain male, blood female and blood male).

A random-effects meta-analysis methodology was selected, the DerSimonian and Laird approach [39], taking in account the expected heterogeneity of the studies we aimed to integrate. The meta-analyses were conducted using the metafor package and following a series of processing steps of the data ([40]. MiRNAs not present in, at least, two of the integrated studies, were removed from the meta-analysis. For the remaining miRNAs, the logarithm of the fold change (LogFC) and its standard error computed on each study were combined for the calculation of the observed expression across all studies. The confidence interval for each LogFC calculated was adjusted for multiple comparisons with the Benjamini and Hochberg method.

For each miRNA meta-analyzed, we determined which studies were involved in the integration and the contribution or weight on the global expression from each of the studies.

**Functional enrichment**

A functional enrichment methodology was carried out on the transcriptomic profile resulting from the four meta-analyses conducted. The aim of this analysis was to establish an association between miRNAs and potentially affected functional effects characteristics. It was necessary to annotate the miRNAs with the genes on which they exert their regulation function to then connect those affected genes with terms linked to them in the Gene Ontology (GO) [41]. The multiMiR library [42] allowed us to obtain the genes targeted by each miRNA analyzed. Afterwards, following the methodology described by García-García [43] and using functions comprised in the mdgsa library [44], we proceeded with the elaboration of a ranked list of genes targeted by the miRNAs under

study, which were subsequently associated with their linked GO terms. The association between genes and GO terms was downloaded from the Biomart database [45]. Statistical significance of the altered functions was adjusted with the BH method.

**Metafun-AD-miRNA web tool**

All data and results generated in the different steps of the meta-analysis are freely available on the Metafun-AD-miRNA platform (http://bioinfo.cipf.es/metafun-AD-miRNA) to any user, allowing the confirmation of obtained results and the exploration of other results of interest. This easy-to-use resource is divided into different sections: i) the summary of analysis results in each phase, followed by detailed results for the ii) exploratory analysis, iii) meta-analysis, and iv) functional profiling for each meta-analysis. The user can interact with the web tool through graphics and tables and explore information associated with specific miRNAs, genes or biological functions.

## Results

The results of this work are organized in four sections: i) the selection of studies resulting from the systematic review, ii) the individual exploratory analysis carried out on each study, iii) the differential expression analysis of miRNA expression in several contrasts, iv) integration of miRNA DE results with a meta-analysis approach and the functional enrichment developed with the GSEA methodology (**Figure 1**).

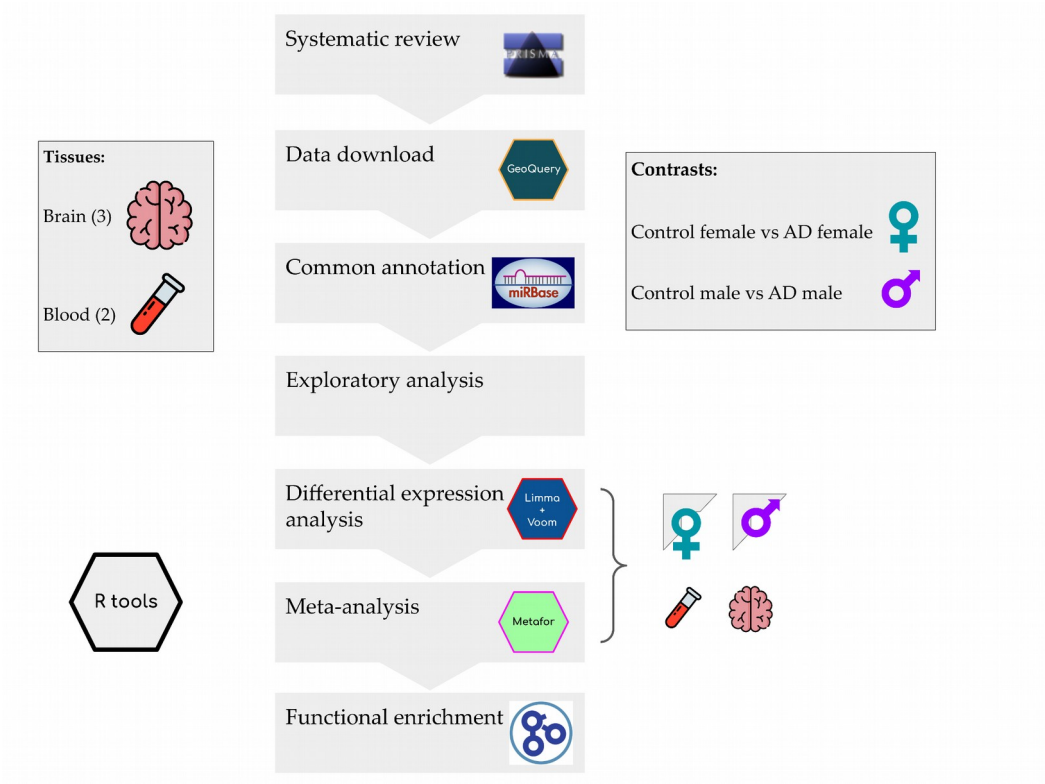


**Figure 1.** Workflow and analysis design. We retrieved relevant studies from GEO-NCBI and ArrayExpress data repositories and performed differential expression analysis on each selected study after data exploration and preprocessing. We performed four different meta-analyses (brain male, brain female, blood male and blood female). Finally, we applied a functional enrichment based on the gene targets of miRNAs profile for each meta-analysis.

**Systematic review**

Following the PRISMA guidelines, we developed a systematic review to identify suitable miRNA focused expression studies in human Alzheimer's disease. Studies must include the sex information associated with each sample. According to the search criteria (**Figure 2**), 27 studies were initially found (26 in GEO and 1 in ArrayExpress). Following removal of duplicates (2), non-human studies (4), not AD focused (9), non-suitable experimental designs (2) and non-miRNA studies (3), 7 studies were selected as initially suitable for the further analysis.

Out of those 7 studies, GSE63501 and GSE153284 were left out of the analysis due to the impossibility of access to the expression data and a lack of standardization of the data, respectively. Therefore, five studies **(Table 1)** were included in the analysis: GSE157239 [46], GSE16759 [47] and GSE48552 [48] analyze the transcriptomics of brain tissue, whereas GSE120584 [49] and GSE46579

[50] are analysis of the miRNA expression in blood of AD patients and healthy controls.

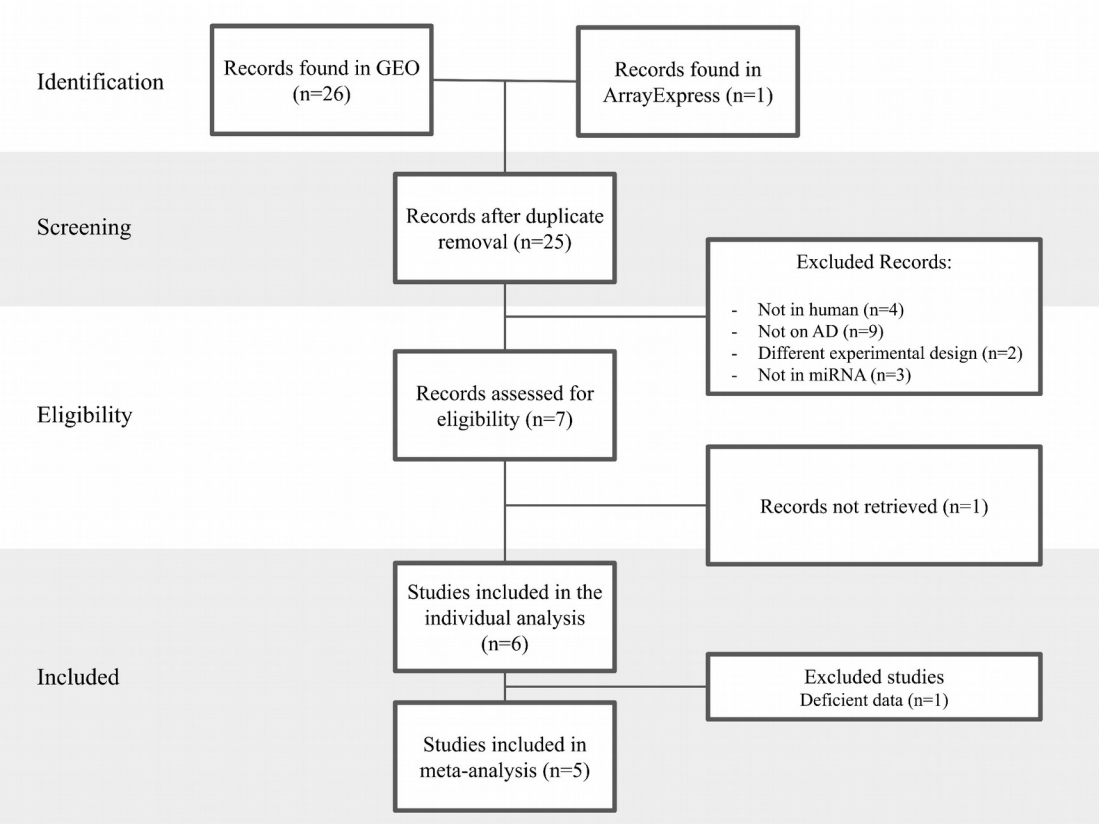


**Figure 2.** PRISMA diagram of the systematic review based on PRISMA guidelines.

**Exploratory analysis and differential expression analysis**

The exploratory analysis allowed us to assess a priori expression patterns in each study, as well as to determine and update the annotation of the miRNAs analyzed. Common miRNAs among studies were determined, and only those miRNAs appearing on two or more studies were considered for the integration analysis. Condition and sex distribution of the samples (**Figure 3**) skewed towards female patients in most of the studies, with less male representation either in the control or AD groups. GSE120584 displayed a much higher sample size than the rest of the selected studies (n=1309).

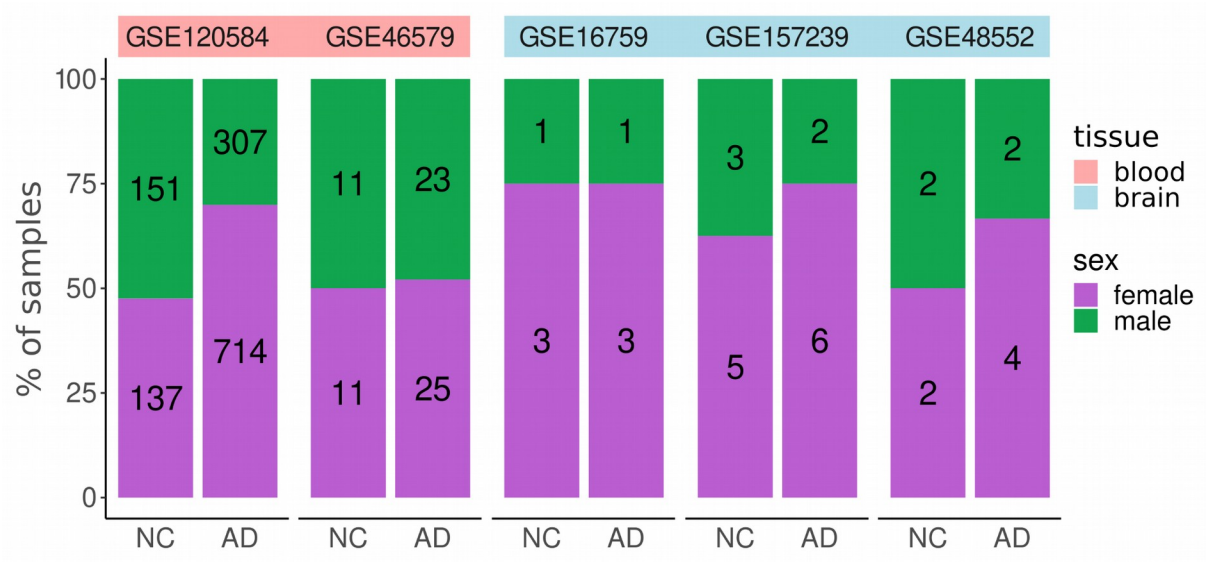


**Figure 3.** Condition, tissue and sex distribution of samples of selected studies.

Expression data distribution showed no anomalous samples, the hierarchical clustering of the samples did not display absolute divisions of the samples based on any of the experimental conditions and neither were bias detected on the PCA visualization.

The differential expression analysis of the individual studies returned altered expression of miRNAs both in females and males in GSE120584, GSE46579 and GSE48552 studies **(Table 2)**. Subsequent analyses focused on the integration of individual differential expression analysis to obtain robust results regarding the alteration of miRNAs for the contrasts considered.

**Meta-analysis and functional enrichment**

Four meta-analyses were performed, integrating the differential expression results of sets of studies based on sample tissue (blood or brain) in females and males **(Table 3)**. For each combination a DL meta-analysis was carried out on the differential expression results from the limma/limma+voom approach. We obtained a combined logFC for each miRNA under analysis and an associated p-value adjusted by BH method.

*Blood meta-analyses*

Considering the meta-analyses based on blood samples, we found significantly altered miRNAs both in females and males (**Figure 4**). In females, all miRNAs significantly altered in AD, 16,

were overexpressed compared to control samples (**Figure 4A**). In males, we found 18 miRNAs overexpressed and 4 miRNAs underexpressed in AD samples compared to control samples (**Figure 4B**). In order to compare the profile of altered miRNAs in males and females, we intersected them (**Figure 4C**) finding 9 common miRNAs altered in both sexes, 7 exclusively affected in females and 13 exclusively altered in males. Thus, we compared the expression profile between males and females of altered miRNAs. MiRNAs altered exclusively in females as well as those altered in males and females shared similar expression patterns in both sexes (**Figure 4D and 4E**). Regarding miRNAs affected in males (**Figure 4F**), their expression profiles were mostly concordant between sexes, except for the mir-145-5p (decreased in males and not significantly increased in females). Afterwards, we compared the target profiles of differentially expressed miRNAs to unveil those genes whose expression would be more affected by AD. Considering females, top targeted genes by overexpressed miRNAs were *ANKRD52* (target of 8 miRNAs), *CELF1* and *LARP1* (targeted by 7 miRNAs), *CBX6*, *KMT2D*, *SETD5*, *SRCAP*, *SRRM2* and *TAOK1* (target of 6 miRNAs). In males, top targeted genes by miRNAs were *LARP1*, *FUS*, *BAZ2A*, *KMT2D* and *DICER1*, whereas *BTBD3*, *NDN*, *NUP43*, *PIK3C2B*, *RAC1*, *RASA1*, *RCAN2*, *RNF38* and *RPRM* were targeted by 3 miRNAs underexpressed in AD.

Based on the miRNA transcriptomic profile obtained, we performed a GSEA on the Biological Process (BP) ontology of Gene Ontology (GO). Remarkably, we found 351 affected BP terms in males (**Supplementary table 1**). From them, 9 BP increased, mainly related to sensory perception related processes, and 342 BP decreased in AD samples compared to control samples (**Figure 4H**). In females AD, the functional enrichment revealed 2 BP downregulated and 4 BP upregulated mostly related to RNA splicing and translation (**Figure 4G**). From them, the most affected BP downregulated in males AD were mostly related to the regulation of mRNA translation, the post-translational modification of proteins, the ubiquitination of proteins and positive regulation of apoptosis (**Figure 4H**).

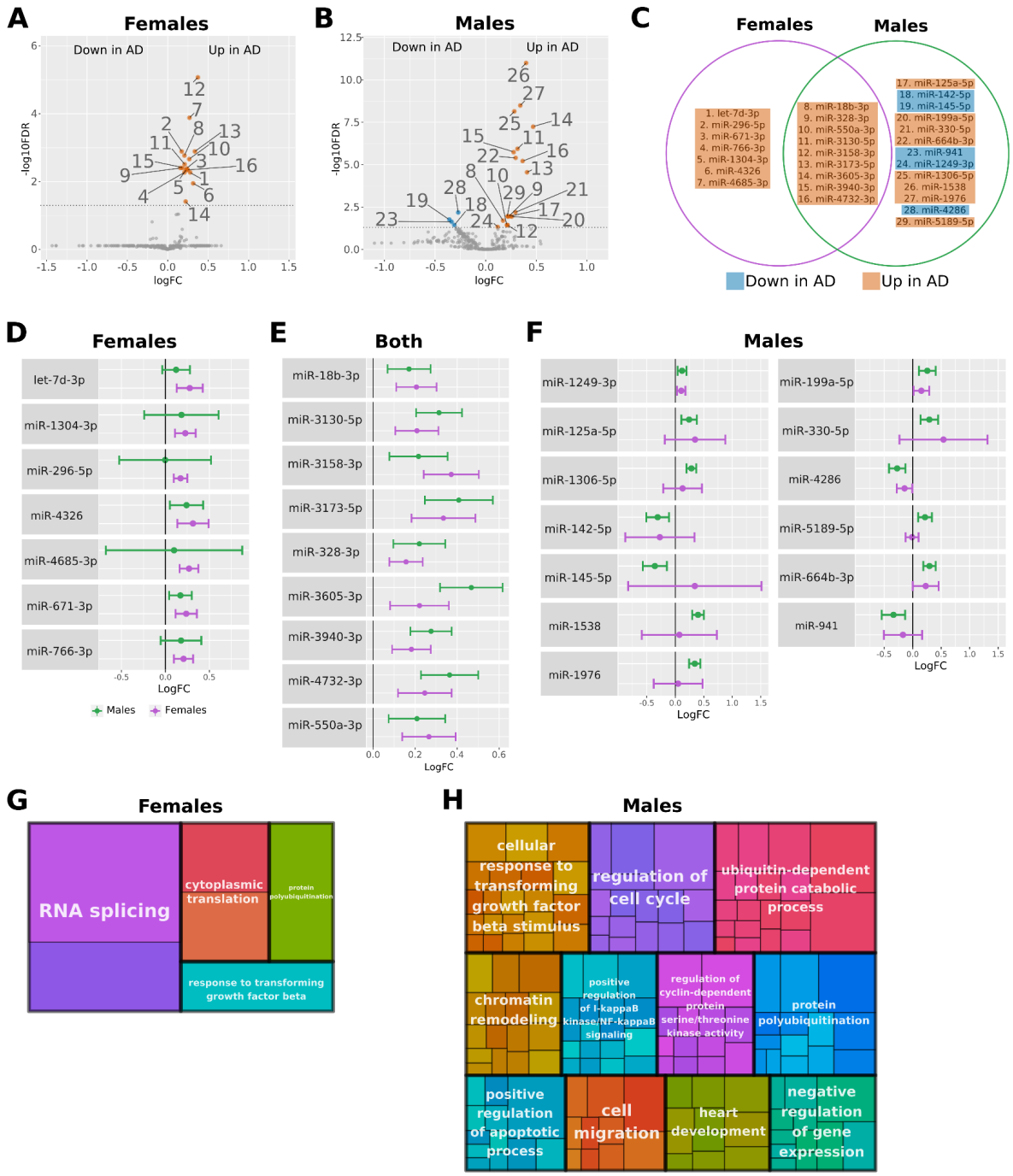


**Figure 4**. Results of blood meta-analyses. **A.** Volcano plot of showing miRNAs overexpressed (red dots, 16 miRNAs) in females. MiRBase IDs corresponding to displayed numbers are listed in panel C. Horizontal dashed gray line indicates -log10(0.05). **B.** Volcano plot of showing miRNAs underexpressed (blue dots, 4 miRNAs) and overexpressed (red dots, 18 miRNAs) in males. MiRBase IDs corresponding to displayed numbers are listed in panel C. Horizontal dashed gray line indicates -log10(0.05). **C.** Venn diagram showing the intersection of DE miRNAs in males and females. **D.** Plot showing the expression profile of miRNAs altered in females. **E.** Plot showing the expression profile of miRNAs altered both in males and females. **D.** Plot showing the expression profile of miRNAs altered in males. **G.** Tree plot of BP terms altered in females. **H.** Tree plot of top 11 clusters of parents BP terms altered in males.

*Brain meta-analyses*

In brain meta-analyses, we found altered expression of miRNAs both in males and females (**Figure 5**). Considering females, 5 miRNAs were underexpressed and 1 miRNA was overexpressed in AD (**Figure 5A**). We explored target genes of differentially expressed miRNAs, finding *CBLN2*, *GOLIM4* and *UHMK1*, targeted by the unique overexpressed miRNA, miR-105-3p. Conversely, *MDM2*, *MTCH2* and *MTRNR2L1* were targeted by 2 underexpressed miRNAs. In males, 2 miRNAs were overexpressed and 2 miRNAs were underexpressed in AD samples compared to control samples (**Figure 5A**). We explored again target genes of differentially expressed miRNAs, finding *NOM1* and *ZNF226* as top targeted genes (2 overexpressed miRNAs) and 71 genes targeted by 2 underexpressed miRNAs (**Supplementary table 2**). The functional enrichment performed on the transcriptomic profiles of females and males did not reveal any affected BP due to AD. Again, we intersected the altered miRNAs between sexes in order to compare their pattern of expression (**Figure 5C**), finding 5 miRNAs exclusively altered in females (**Figure 5D**), 3 miRNAs solely affected in males (**Figure 5E**) and a common miRNA underexpressed in both sexes (miR-767-5p, **Figure 5F**).

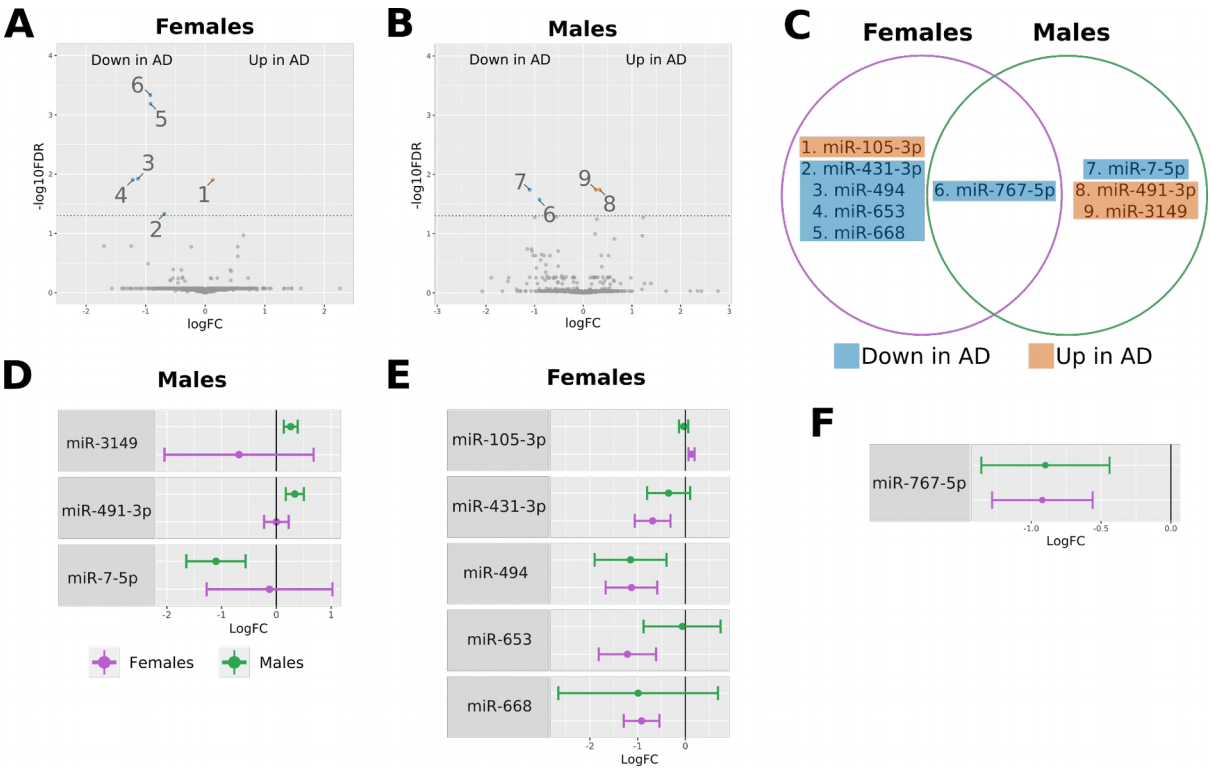

**Figure 5**. Results of brain meta-analyses. **A.** Volcano plot of showing miRNAs underexpressed (blue dots, 2 miRNAs) and overexpressed (red dots, 2 miRNAs) in males. MiRBase IDs corresponding to displayed numbers are listed in panel C. Horizontal dashed gray line indicates -log10(0.05). **B.** Volcano plot of showing miRNAs underexpressed (blue dots, 5 miRNAs) and overexpressed (red dot, 1 miRNAs) in females. MiRBase IDs corresponding to displayed numbers are listed in panel C. Horizontal dashed gray line indicates -log10(0.05). **C.** Venn diagram showing the intersection of DE miRNAs in males and females. **D.** Forest plot of miRNAs altered both in males and females.

*Sex-based intersections of altered miRNAs*

We compared those miRNAs differentially expressed in brain and blood of females and males. In females, we found (using raw p value < 0.05) that let-7d-3p and miR-486-5p were altered, being overexpressed in AD for both tissues in let-7d-3p and up in AD in brain and down in AD in blood for miR-486-5p. In males, we found alterations for 5 miRNAs using the same approach: miR-7-5p and miR181a-5p were down in both tissues, miR-145-5p was down in blood and up in brain, miR-2110 was up in blood and down in brain and miR-664a-3p was up in both tissues.

## Discussion

Despite the multiple sex differences described in AD symptomatology and epidemiology, their molecular basis remains unclear. Considering and understanding the impact of sex differences in the research of diseases is crucial to improve clinical outcomes [51]. Furthermore, miRNAs are important regulators of gene expression and their relevance in AD is being increasingly studied as reviewed in [22,52,53]. We conducted a systematic review and different meta-analyses to unveil sex-based miRNA profiles in blood and brain of AD patients. In general, the selected studies showed a higher representation of female AD samples according to epidemiology described [54]. Our results show similar effects of AD in males and females in both tissues. Moreover, the miRNAs commonly affected in both sexes are altered in the same way and could be studied further as potential biomarkers of AD. Nevertheless, the functional enrichment of miRNAs in blood revealed sex-specific BP altered.

*Blood meta-analyses*

We found several deregulated miRNAs in females and males AD. The intersection analysis of these miRNAs to deeper explore sex differences and similarities point to sex-specific altered miRNAS, as well as miRNAs commonly affected in both sexes that are altered in the same way (upregulated in AD) and could be studied further as potential biomarkers of AD.

In this work, we reported a 7-miRNA signature altered in female AD patients, most of them previously unrelated to AD. Solely let-7d-3p and miR-671-3p have been previously reported as overexpressed in AD patients in blood meta-analyses without sex perspective [50,55] 550a-3p (up) and 671-3p (up). From the 13 miRNAs panel specific for males, miR-1306-5p (up) expression alteration was previously related to AD in another meta-analysis [56] and appeared downregulated in extracellular vesicles of AD patients [57], which do no contradict that miR-1306-5p can be up in blood as free circulant miRNA. Besides, the role of miR-1306-5p in AD has been also explored in cell cultures [58]. We also found a significant decrease in miR-142-5p expression of male AD patients, related to spatial learning and memory in AD animal models [59,60][60,61] our results suggest that miR-142-5p may be a potential target in AD for the improvement in synaptic signaling." Considering most targeted genes of altered miRNAs in females, the nuclear speckle scaffold protein SRRM2 is accumulated in neuron cytoplasm of AD patients [62–64 ]and TAOK1 phosphorylation induces the formation of neurofibrillary tangles [65]. The rest of top targeted genes were mostly related to gene expression and chromatin organization, but not linked to AD progression.

We also analysed the functional effects of miRNAs' altered expression based on their targets, since a single miRNA can regulate the expression of multiple genes, resulting in complex interaction networks [66,67]. Females and males showed different functional profiles affected by deregulated miRNAs. In females, splicing and translation were primarily altered by deregulated miRNAs. Splicing is key to generating new protein variants and dysregulation in tau splicing has been associated with neurodegenerative diseases and dementia [68]. Moreover, altered translation could influence the proteostasis and the accumulation of proteins in cytoplasm contributing importantly to neuroinflammation and neurodegeneration [69,70]. However in males, deregulated miRNA influence

processes linked to sensory perception related processes (kappa signaling, stimulus by growth factors, cyclin and kinases…) and ubiquitination and catabolic processes that can influence accumulation of misfolded proteins, hallmark of AD.

*Brain meta-analyses*

Brain meta-analyses revealed alterations in the expression of different miRNAs in females and males. The intersection of these results revealed miR-767-5p commonly overexpressed in females and males with AD, supporting a previous report that suggested it as a biomarker candidate in the cerebrospinal fluid of AD patients [71]. Regarding sex-specific results, in females none of the significantly deregulated miRNAs reported have been previously linked to AD, except the miR-494, involved in stress pathways in AD [72]. In males, miR-7-5p was underexpressed in AD patients, contrasting with a previous study that reported an increased expression linked to NLRP3 inflammasome [73]. MiR491-3P and miR3149, both increased in brain samples of males, were not previously linked to AD. MDM2 gene, hypothetically overexpressed in females based on the reduced expression of miRNAs that target it, regulates p53 degradation and has been previously linked to AD. The rest of top affected genes are mostly related to gene expression and translation in general terms, but not specifically linked to AD.

**Strengths and limitations**

We performed an in silico strategy to evaluate and integrate the differential expression of miRNA transcriptomic studies. Previous systematic reviews and meta-analyses have been performed on this subject [56,74,75]. Nonetheless, we performed, to the best of our knowledge, the first systematic review with sex perspective to unveil the specific effects of AD on miRNA expression profiles of different tissues in females and males. Our approach allowed the analysis of sex-based miRNA consensus differential expression profiles of males and females, highlighting a partial overlapping in the miRNAs altered by AD progression, specially in blood samples.

Limitations, low sample size compared to previously mentioned studies due to lack of sex info or studies conducted just on one sex; disbalance between sexes in sample size could explain differences in found altered miRNAs. Finally, important covariates such as medication usage, years of disease after diagnosis, and post-mortem interval were not included in the metadata of most original studies, thus increasing the unexplained variability of the data.

**Perspectives and significance**

The results described highlight a different sex-based impact of AD in brain and blood samples. Together, our results outline specific alterations in miRNAs expression profile in these two tissues. Considering sex differences, we describe a partial overlapping of miRNAs altered in AD samples of males and females, especially in blood samples. Nevertheless, we also found sex-specific alterations of miRNAs expression, highlighting the sex-based differential impact of AD on the regulation of gene expression. Thus, the present study takes a novel approach to assess the sex-based differences in miRNAs expression of AD patients through a comprehensive bioinformatic strategy.

## Conclusions

In conclusion, our in silico approach has identified alterations in the expression of specific and shared miRNAs for male and female AD patients that represent potential candidates as biomarkers to diagnosis, especially promising in blood samples. Moreover, we have identified differential functional alterations as a consequence of AD progression in blood samples, being mainly related to RNA processing and translation in females, and regulation of kinase activity, chromatin remodeling and ubiquitination processes in males. These findings aim to better understand the role of miRNA expression in AD progression, emphasizing differences and similarities found in males and females. Finally, we stress the key role of open data sharing for scientific advancement.

## Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and materials

The data used for the analyses described in this work are publicly available at GEO [34]. The accession numbers of the GEO datasets downloaded are GSE16759, GSE46579, GSE48552, GSE120584 and GSE157239. The accession numbers of the ArrayExpress datasets downloaded are E-MTAB-1194 and E-MEXP-1416.

### Competing interests

The authors declare that they have no competing interests

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### Authors contributions

JLO and HC analyzed the data; FGG designed and supervised the bioinformatics analysis; HC and MRH designed and implemented the web tool; JLO, HC and FGG wrote the manuscript; ZA, ISS, FG, MIV, FRG, AM, RM, BR and FGG helped in the interpretation of the results; HC, ZA, MIV, ISS, FRG, BR, AM, RM and FGG writing-review and editing; FGG conceived the work. All authors read and approved the final manuscript.

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## Tables

**Table 1.** Summary of the selected studies from the systematic review.

| ID | Study type | Platform | Samples | Control | Cases | Type of sample | PMID |
|---|---|---|---|---|---|---|---|
| GSE157239 | Non-coding RNA profiling by array | GPL21572 | 16 | 8 | 8 | Brain (Temporal cortex) | 32920076 |
| GSE16759 | Non-coding RNA profiling by array | GPL8757 | 8 | 4 | 4 | Brain (Parietal lobe) | 20126538 |
| GSE48552 | Non-coding RNA profiling by high throughput sequencing | GPL11154 | 12 | 6 | 6 | Brain (Prefrontal cortex) | 24014289 |
| GSE120584 | Non-coding RNA profiling by array | GPL21263 | 1309 | 288 | 1021 | Blood (serum) | 34686734 |
| GSE46579 | Non-coding RNA profiling by high throughput sequencing | GPL11154 | 70 | 22 | 48 | Blood (whole blood) | 23895045 |

**Table 2.** Differentially expressed miRNAs by study and by contrast.

| Tissue | Study | miRNAs analysed | Female contrast | | Male contrast | |
|---|---|---|---|---|---|---|
| | | | **Upregulated** | **Downregulated** | **Upregulated** | **Downregulated** |
| Brain | GSE157239 | 2561 | 0 | 0 | 0 | 0 |
| | GSE16759 | 462 | 0 | 0 | 0 | 0 |
| | GSE48552 | 832 | 88 | 79 | 34 | 7 |
| Blood | GSE120584 | 2521 | 404 | 47 | 491 | 553 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | GSE46579 | 326 | 37 | 58 | 7 | 16 |

**Table 3.** Results of differential expression meta-analyses based on individual studies.

| Tissue | miRNAs meta-analysed | Female contrast | | Male contrast | |
|---|---|---|---|---|---|
| | | **Upregulated** | **Downregulated** | **Upregulated** | **Downregulated** |
| Brain | 850 | 1 | 5 | 2 | 2 |
| Blood | 314 | 16 | 0 | 18 | 4 |