Emerging blood exosome-based biomarkers for preclinical and clinical Alzheimer's disease: a meta-analysis and systematic review

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From the Contents

Abstract

Blood exosomes, which are extracellular vesicles secreted by living cells into the circulating blood, are regarded as a relatively noninvasive novel tool for monitoring brain physiology and disease states. An increasing number of blood cargo-loaded exosomes are emerging as potential biomarkers for preclinical and clinical Alzheimer's disease. Therefore, we conducted a meta-analysis and systematic review of molecular biomarkers derived from blood exosomes to comprehensively analyze their diagnostic performance in preclinical Alzheimer's disease, mild cognitive impairment, and Alzheimer's disease. We performed a literature search in PubMed, Web of Science, Embase, and Cochrane Library from their inception to August 15, 2020. The research subjects mainly included Alzheimer's disease, mild cognitive impairment, and preclinical Alzheimer's disease. We identified 34 observational studies, of which 15 were included in the quantitative analysis (Newcastle-Ottawa Scale score 5.87 points) and 19 were used in the qualitative analysis. The meta-analysis results showed that core biomarkers including $A\beta_{1-42}$, P-T181-tau, P-S396-tau, and T-tau were increased in blood neuron-derived exosomes of preclinical Alzheimer's disease, mild cognitive impairment, and Alzheimer's disease patients. Molecules related to additional risk factors that are involved in neuroinflammation (C1q), metabolism disorder (P-S312-IRS-1), neurotrophic deficiency (HGF), vascular injury (VEGF-D), and autophagy-lysosomal system dysfunction (cathepsin D) were also increased. At the gene level, the differential expression of transcription-related factors (REST) and microRNAs (miR-132) also affects RNA splicing, transport, and translation. These pathological changes contribute to neural loss and synaptic dysfunction. The data confirm that the above-mentioned core molecules and additional risk-related factors in blood exosomes can serve as candidate biomarkers for preclinical and clinical Alzheimer's disease. These findings support further development of exosome biomarkers for a clinical blood test for Alzheimer's disease. This meta-analysis was registered at the International Prospective Register of Systematic Reviews (Registration No. CRD4200173498, 28/04/2020).

Key Words: Alzheimer's disease; amyloid-β; biomarkers; blood; exosomes; extracellular vesicles; meta-analysis; mild cognitive impairment; systematic review; tau protein

Introduction

Alzheimer's disease (AD) is a common neurodegenerative disease characterized by a long preclinical phase with progressively irreversible pathology (Scheltens et al., 2016). In China, the prevalence of dementia is estimated to be 6.0% in people aged 60 years or older, among whom 983 million have AD (Jia et al., 2020b). The occurrence and development of AD are due to multiple factors (Jack et al., 2018). Core pathological features, including amyloid-β (Aβ) deposition and tau hyperphosphorylation, emerge at a younger age of ~45 years (Robinson et al., 2017). Moreover, additional factors, such as inflammation, oxidation, nutritional deficiency, metabolic disorder, and differential RNA expression further induce the pathological development of AD. The interactions of core pathological factors and additional risk factors aggravate neurodegeneration and neural synaptic dysfunction during the development of AD (Tiwari et al., 2019; Guo et al., 2020b). It is generally believed the pathophysiological changes in AD begin at least 10 years prior to the stage of clinically diagnosable symptoms (Fiandaca et al., 2015). Therefore, discovering core and additional pathogenic biomarkers is essential for identifying patients with preclinical AD and for developing more effective therapies.

Cumulative evidence supports the notion that the biomarkers, $A\beta_{1-42}$, total tau (T-tau), and phosphorylated tau (P-tau), in the cerebrospinal fluid (CSF) reflect the typical pathological signature of AD (Guo et al., 2020b). Furthermore, molecular imaging by positron emission tomography revealed that Aβ burden and tau accumulation in clinical AD patients are greater than those in clinically normal controls (Mattsson-Carlgren et al., 2020; Milà-Alomà et al., 2020). Importantly, the same results of CSF biomarkers and imaging of biomarkers were found in early preclinical AD and mild cognitive impairment (MCI) patients (Olsson et al., 2016).

Although great progress has been made with CSF biomarkers and molecular imaging, it is necessary to find biomarkers that can be part of clinical practice and are inexpensive, widely available, and noninvasive; this can only be achieved by blood biomarkers (Solje et al., 2021). It is widely accepted that the development of reliable predictive blood biomarkers is crucial in the pursuit of disease-modifying interventions for AD and their widespread implementation (Lewczuk et al., 2018). However, there are no consistent results in the studies of blood biomarkers for AD. Previous studies have shown that the ratios of amyloid precursor protein 669–711 ($APP_{669-711}$)/$A\beta_{1-42}$ and $A\beta_{1-40}$/$A\beta_{1-42}$ were increased in the blood of MCI and AD patients (Nakamura et al., 2018), while in a large-scale meta-analysis, T-tau has been reported to be remarkably increased in the blood of AD patients, but $A\beta_{1-42}$ and $A\beta_{1-40}$ remained unchanged (Olsson et al., 2016). Notably, the concentrations of $A\beta_{1-42}$, T-tau, and P-tau in blood exosomes have been shown to distinguish AD cohorts from clinically normal controls or other types of dementia. Moreover, the abnormal changes could be detected more than 10 years prior to the

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clinical onset of AD with a similar sensitivity to CSF in diagnosing AD and MCI (Fiandaca et al., 2015; Jia et al., 2019). Exosomes are membrane-bound particles shed by most cell types and are important mediators of cell-cell communication by delivering their cargo, which can reflect brain physiology and disease states (Kalluri and LeBleu, 2020). Recent studies have shown several novel candidate molecular biomarkers in neuron-derived exosomes (NDE) and astrocyte-derived exosomes (ADE) in the blood that were significantly associated with AD (Goetzl et al., 2016a, b, 2018a; Hornung et al., 2020; Serpente et al., 2020). Blood exosome-derived neurogranin, a neuron-specific and post-synaptic protein, can be used as a cognitive biomarker for AD (Liu et al., 2020). Importantly, numerous studies have consistently demonstrated that these blood exosome-derived molecular biomarkers can also be applied for the diagnosis of early preclinical AD and MCI (Ellegaard et al., 2020; Eren et al., 2020; Hornung et al., 2020; Wang and Zhang, 2020).

A recent study has analyzed the diagnostic ability of exosome-derived contents, but it did not verify the diagnostic efficacy of each biomarker in detail (Xing et al., 2021). Another study has also explored the possibility of brain-derived exosomal proteins as AD biomarkers (Kim et al., 2021). Compared with previous studies, in this review, we included more relevant studies that were limited to proteins in exosomes and to small molecules derived from exosomes, such as microRNA (miRNA). Additionally, we aimed to perform a more comprehensive meta-analysis and systematic review to identify candidate molecular biomarkers derived from blood exosomes for monitoring core and additional disease pathogeneses and to reveal those that can be used as effective diagnostic biomarkers of preclinical AD, MCI, and AD.

Data and Methods

This meta-analysis and systematic review were reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and the Observational Studies in Epidemiology (MOOSE) checklist (Stroup et al., 2000; Moher et al., 2009) and was registered at the International Prospective Register of Systematic Reviews (https://www.crd. york.ac.uk/PROSPERO/) (number CRD42020173498).

Search strategy

Two investigators (HWL and YY) independently performed a literature search in PubMed, Web of Science, Embase, and Cochrane Library from their inception to August 15, 2020. The last time we conducted a literature search was August 15, 2020. We also did a manual search by checking the reference lists of key published articles and imposed no language restrictions. Moreover, we considered all potentially eligible studies for review, irrespective of the primary outcome or study type.

The detailed search terms were as follows:

PubMed database retrieval strategy:

#1 exosome [All fields]
#2 exosomes [All fields]
#3 extracellular vesicles [All fields]
#4 EVs [All fields]
#5 micro vesicles [All fields]
#6 micro-vesicles [All fields]
#7 microvesicles [All fields]
#8 MVs [All fields]
#9 secretory vesicles [All fields]
#10 cell-derived microparticles [All fields]
#11 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10
#12 blood [All fields]
#13 plasma [All fields]
#14 serum [All fields]
#15 #12 OR #13 OR #14
#16 Alzheimer's disease [All fields]
#17 mild cognitive impairment [All fields]
#18 preclinical Alzheimer's disease [All fields]
#19 #16 OR #17 OR #18
#20 #11 AND #15 AND #19

Web of Science database retrieval strategy:

#1 TS=(exosome)
#2 TS=(exosomes)
#3 TS=(extracellular vesicles)
#4 TS=(EVs)
#5 TS=(micro vesicles)
#6 TS=(micro-vesicles)
#7 TS=(microvesicles)
#8 TS=(MVs)
#9 TS=(secretory vesicles)
#10 TS=(cell-derived microparticles)
#11 (((((((((#1) OR #2) OR #3) OR #4) OR #5) OR #6) OR #7) OR #8) OR #9) OR #10
#12 TS=(blood)
#13 TS=(plasma)
#14 TS=(serum)
#15 ((#12) OR #13) OR #14
#16 TS=(Alzheimer's disease)
#17 TS=(mild cognitive impairment)
#18 TS=(preclinical Alzheimer's disease)
#19 ((#16) OR #17) OR #18)
#20 ((#11 AND #15) AND #19)

Cochrane Library database retrieval strategy:

#1 (exosome): ti, ab, kw
#2 (exosomes): ti, ab, kw
#3 (extracellular vesicles): ti, ab, kw
#4 (EVs): ti, ab, kw
#5 (micro vesicles): ti, ab, kw
#6 (micro-vesicles): ti, ab, kw
#7 (microvesicles): ti, ab, kw
#8 (MVs): ti, ab, kw
#9 (secretory vesicles): ti, ab, kw
#10 (cell-derived microparticles): ti, ab, kw
#11 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10
#12 (blood): ti, ab, kw
#13 (plasma): ti, ab, kw
#14 (serum): ti, ab, kw
#15 #12 OR #13 OR #14
#16 (Alzheimer's disease): ti, ab, kw
#17 (mild cognitive impairment): ti, ab, kw
#18 (preclinical Alzheimer's disease): ti, ab, kw
#19 #16 OR #17 OR #18
#20 #11 AND #15 AND #19

Selection criteria

The included articles met the following criteria: (1) the reported data of exome-derived cargos included at least one of these three groups - AD, MCI, and preclinical AD - and healthy control subjects; (2) the diagnosis of AD and MCI was consistent with the 1984 NINCDS-ADRDA Criteria (Dubois et al., 2007), the 2011 NIA-AA Criteria (Albert et al., 2011; McKhann et al., 2011), the 2014 IWG-2 Criteria (Dubois et al., 2014), or the 2018 AT (N) framework (Jack et al., 2018), and the preclinical AD is a group of people with normal cognitive but positive pathological symptoms of AD (Fiandaca et al., 2015); (3) exosomes were from peripheral blood, serum, or plasma, and could originate from different cell types; (4) we had no restrictions on the study type, language, and intervention, but the articles were officially published before August 15, 2020.

The exclusion criteria were: (1) clinical studies, animal experiments, or reviews reporting the outcome of exosomes; (2) articles reporting the levels of biomarkers in exosomes of participants with AD, MCI, or preclinical AD, without a healthy control group.

Data extraction

Two individuals (HWL and HHL) extracted the original data from the published articles or the table in the Supplementary Materials and organized it in Excel, which was then checked by a third person (YY). For research where there was no specific data in the original text, we tried to email the author. The extracted details included the study design, country, publication year, participant characteristics (name, sample size, gender, and age), the Mini-Mental State Examination (MMSE) score, biomarkers, the source of the exosomes and the method of isolation and quantification, and the mean ± standard deviation (SD)/standard error of mean (SEM) of the cargos' concentration in exosomes and extracellular vesicles (EVs). Additionally, before conducting the meta-analysis, we merged the multiple sets of non-crossing subgroup data of several studies according to the latest Cochrane Handbook for Systematic Reviews of Interventions (Cumpston et al., 2019).

Assessment of methodological quality

We assessed the quality of each study using the Newcastle-Ottawa Scale (NOS) (Stang, 2010). The NOS ranges from 0 to 10 stars. Each study was judged according to three broad criteria: selection of participants (4 points), comparability of studies (2 points), and ascertainment of outcome of interest (4 points). A score of 0–3 points was considered a low-quality study, a score of 4–6 points equated to a moderate quality study, and a score of 7–10 points was considered a high quality study (Neal et al., 2019).

Embase database retrieval strategy:

#1 'exosome'/exp OR exosome

#2 'exosomes'/exp OR exosomes

#3 'extracellular vesicles'/exp OR 'extracellular vesicles' OR (extracellular AND ('vesicles'/exp OR vesicles))

#4 'EVs'

#5 'micro vesicles' OR (micro AND ('vesicles'/exp OR vesicles))

#6 'micro vesicles'

#7 'microvesicles'

#8 'MVs'

#9 'secretory vesicles'/exp OR 'secretory vesicles' OR (secretory AND ('vesicles'/exp OR vesicles))

#10 'cell-derived microparticles'/exp OR 'cell-derived microparticles' OR ('cell derived' AND ('microparticles'/exp OR microparticles))

#11 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10

#12 'blood'/exp OR blood

#13 'plasma'/exp OR plasma

#14 'serum'/exp OR serum

#15 #12 OR #13 OR #14

#16 'alzheimer disease'/exp OR 'alzheimer disease' OR (alzheimer AND ('disease'/exp OR disease))

#17 'mild cognitive impairment'/exp OR 'mild cognitive impairment' OR (mild AND cognitive AND ('impairment'/exp OR impairment))

#18 'preclinical alzheimer disease' OR (preclinical AND alzheimer AND ('disease'/exp OR disease))

#19 #16 OR #17 OR #18

Outcomes

In the data we extracted, the author, year, country, study group, sample size, sex, age, MMSE scores, detected biomarkers, sample source, isolation methods, analytical methods, and study type were regarded as baseline information, which is summarized in **Additional Table 1**. Core pathological biomarkers were regarded as primary outcomes, and biomarkers reflecting other risk factors were regarded as secondary outcomes.

Statistical analysis

We used the Stata 16.0 software (StataCorp LLC, College Station, TX, USA) to perform all statistical analysis. First, we used manual scoring to evaluate the quality of the included studies according to the NOS. Sample sizes (mean ± SD) were primarily used to generate effective sizes, and in some articles, where data were expressed as the mean ± SEM, we converted the data to mean ± SD for meta-analysis. Then, we calculated standardized mean difference (SMD) and 95% confidence intervals (CIs) and generated forest plots to compare the mean cargo concentrations in exosomes between patients with AD, MCI, or preclinical AD and healthy control subjects to eliminate the effects of different extraction and measurement methods and different dimensions. We performed a fixed effects model using the inverse-variance method, when there was no evidence of heterogeneity between studies. Otherwise, we chose the random effects model using the Der Simonian and Laird method for meta-analysis as a statistical model (DerSimonian and Laird, 2015). In the next statistical analysis, we performed overall meta-analysis between AD or MCI patients and healthy control subjects. The heterogeneity was assessed by Q test and I^2 index. The statistical significance of the Q test was set at $P < 0.1$, and the I^2 index was used to evaluate the degree of heterogeneity, with 25%, 50%, and 75% suggesting low, moderate, and high levels of heterogeneity, respectively. For some highly heterogeneous results, sensitivity analysis rather than subgroup analysis was further performed to explore the source of heterogeneity, because fewer studies were included. Furthermore, considering there were few articles included in the individual quantitative analysis, we did not analyze publication bias. Statistical significance was considered at $P < 0.05$, except where noted, and $P < 0.1$ was regarded as a trend.

Results

Searching results and characteristics of studies

We searched 1190 articles and screened 940 articles after duplication. After reading the titles and abstracts, 53 articles were considered as potentially eligible. After reviewing the full text, 19 articles were excluded and 34 articles were included in this meta-analysis and systematic review (Cheng et al., 2015; Fiandaca et al., 2015; Goetzl et al., 2015a, b, 2016a, b, 2018a, b, 2019; Winston et al., 2016, 2018, 2019; Kapogiannis et al., 2015; Lugli et al., 2015; Yang et al., 2018; Agliardi et al., 2019; Cha et al., 2019; Gamez-Valero et al., 2019; Haddad et al., 2019; Jia et al., 2019, 2020; Kapogiannis et al., 2019; Aharon et al., 2020; Ellegaard et al., 2020; Gu et al., 2020; Li et al., 2020a; Nam et al., 2020; Nie et al., 2020; Perrotte et al., 2020; Serpente et al., 2020; Sun et al., 2020; Wang et al., 2020; Zhang et al., 2020; Zhao et al., 2020). Of these, 15 articles were finally included in the quantitative analysis (Fiandaca et al., 2015; Goetzl et al., 2015b, 2016a, b, 2018a; Winston et al., 2016; Winston et al., 2018; Agliardi et al., 2019; Jia et al., 2019; Winston et al., 2019; Gu et al., 2020; Jia et al., 2020a; Nam et al., 2020; Zhao et al., 2020) and 19 articles were used for the qualitative analysis (Cheng et al., 2015; Goetzl et al., 2015a, 2018b, 2019; Kapogiannis et al., 2015; Lugli et al., 2015; Yang et al., 2018; Cha et al., 2019; Gamez-Valero et al., 2019; Haddad et al., 2019; Kapogiannis et al., 2019; Aharon et al., 2020; Ellegaard et al., 2020; Nie et al., 2020; Perrotte et al., 2020; Serpente et al., 2020; Sun et al., 2020; Wang et al., 2020; Zhang et al., 2020). The study selection process is shown in **Figure 1**. The detailed characteristics of the included studies are shown in **Additional Table 1**. The final sample size consisted of 4117 individuals (AD: 1423, MCI: 640, preclinical AD: 300, and healthy controls: 1754). Ten of these studies were from China, 16 were from the United States, and the rest were from different countries, including Israel, Australia, Denmark, Germany, South Korea, Canada, and Italy. Additionally, among the included studies, 28 studies used commercial kits to extract and isolate exosomes, five studies used ultracentrifugation, one study used size exclusion chromatography, 22 studies used traditional enzyme-linked immunosorbent assay technology, 10 studies used PCR to quantify exosomal cargo, and two studies used high-sensitivity and high-throughput techniques of mesoscale discovery and the Luminex system.

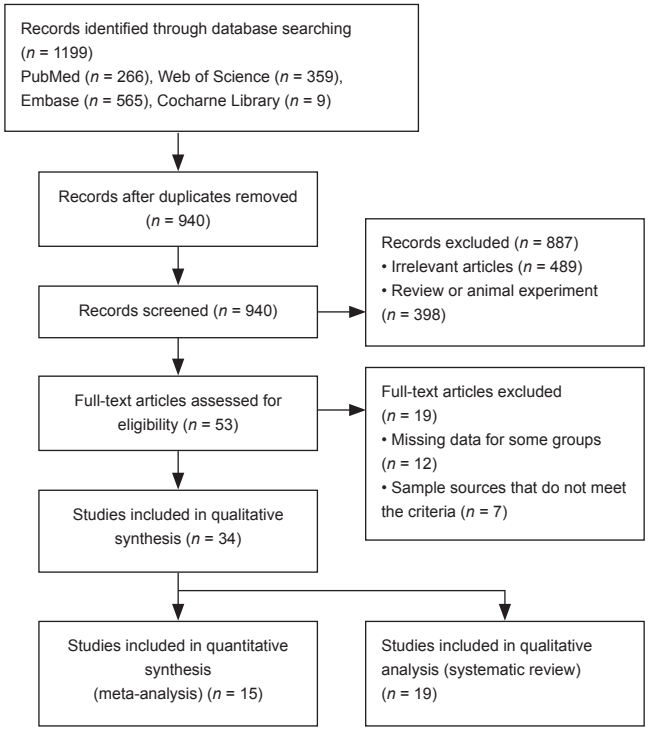

Figure 1 | **Flow diagram of the study selection process.**

The quality assessment results (**Table 1**) showed that the average quality of the 15 studies included in the meta-analysis was 5.87 points, which was considered medium quality.

Blood exosomal $A\beta_{1-42}$, APP cleaving enzymes, and soluble APPs in AD, MCI, and preclinical AD patients

Nine studies were designed to compare the level of blood NDE $A\beta_{1-42}$ in 387 AD patients, 340 MCI patients, and 414 healthy control subjects (Fiandaca et al., 2015; Goetzl et al., 2016a; Winston et al., 2016, 2018; Jia et al., 2019; Gu et al., 2020; Li et al., 2020a; Nam et al., 2020; Zhao et al., 2020). The level of blood NDE $A\beta_{1-42}$ was significantly higher in 359 AD patients and 311 MCI patients than in 338 healthy control subjects (SMD = 2.37, 95% CI: 1.58–3.15, $P < 0.001$; **Figure 2A**) (Fiandaca et al., 2015; Goetzl et al., 2016a; Winston et al., 2016; Jia et al., 2019; Gu et al., 2020; Li et al., 2020a; Nam et al., 2020; Zhao et al., 2020) and 308 healthy control subjects (SMD = 3.05, 95% CI: 1.66–4.45, $P < 0.001$; **Figure 2B**) (Fiandaca et al., 2015; Winston et al., 2016, 2018; Li et al., 2020a; Nam et al., 2020; Zhao et al., 2020), respectively. Simultaneously, we compared the level of blood NDE $A\beta_{1-42}$ between 263 AD patients and 279 MCI patients (Fiandaca et al., 2015; Winston et al., 2016; Jia et al., 2019; Li et al., 2020a; Nam et al., 2020; Zhao et al., 2020) and found no statistically significant difference (SMD = 0.71, 95% CI: –0.12–1.54, $P = 0.09$; **Figure 2C**), though a rising trend was observed in the AD patients. Additionally, no specific data were reported in two studies (Perrotte et al., 2020; Sun et al., 2020), but the results showed that the blood NDE $A\beta_{1-42}$ in the AD patients was significantly higher than that in the healthy control subjects, with different concentrations at different AD stages. One study reported that the blood NDE $A\beta_{1-42}$ level in patients with preclinical AD was significantly higher than that in healthy control subjects, and significantly lower than that in AD patients (Fiandaca et al., 2015). In the study by Wang et al. (2020), the level of blood exosomes containing β-secretase 1 (BACE-1)-antisense transcript (BACE1-AS) in AD patients was reported to be significantly higher than that in healthy controls. Inconsistent with blood NDE, the level of BACE-1 in blood ADE in AD patients was significantly higher than that in

Table 1 | **Quality assessment of studies by the Newcastle-Ottawa Scale (NOS)**

Author, year	Selection				Comparability		Exposure				Total scores
	S1	S2	S3	S4	C1a	C1b	E1a	E1b	E2	E3	
Fiandaca et al., 2015	*	*	–	*	*	*	–	–	*	–	6
Goetzl-2 et al., 2015	*	*	–	*	*	*	–	–	*	–	6
Goetzl-4 et al., 2016	*	*	–	*	*	*	–	*	*	–	7
Goetzl-5 et al., 2016	*	*	–	*	*	*	–	–	*	–	6
Winston-1 et al., 2016	*	*	–	*	*	–	–	–	*	–	5
Goetzl-7 et al., 2018	*	*	–	*	*	*	–	*	*	–	7
Winston-2 et al., 2018	*	*	–	*	*	–	–	*	*	–	6
Agliardi et al., 2019	*	–	–	*	*	*	–	–	*	–	5
Jia-1 et al., 2019	*	*	–	*	*	*	–	–	*	–	6
Winston-3 et al., 2019	*	*	–	*	*	*	–	–	*	–	6
Gu et al., 2020	*	–	–	*	*	-	–	*	*	–	5
Jia-2 et al., 2020	*	*	–	*	*	*	–	–	*	–	6
Li et al., 2020	*	–	–	*	*	*	–	–	*	–	5
Nam et al., 2020	*	*	–	*	*	–	–	*	*	–	6
Zhao et al., 2020	*	*	–	*	*	*	–	–	*	–	6

The NOS was used for the quality assessment of observational studies, which has three large modules (Selection, Comparability, Exposure) and eight small questions. The sign after the author means that there are several authors with the same last name or some studies with the same first author, and the number represents the order of the article, which aims to distinguish these studies. S1: Is the case definition adequate; S2: representativeness of the cases; S3: selection of controls; S4: definition of controls; C1a: comparability of cases and controls on the basis of the design or analysis – Choice a) study controls for (select the most important factor.); C1b: comparability of cases and controls on the basis of the design or analysis – Choice b) study controls for any additional factor (This criteria could be modified to indicate specific control for a second important factor.); E1a: ascertainment of exposure – Choice a) secure record (e.g. surgical records); E1b: ascertainment of exposure – Choice b) structured interview where blind to case/control status; E2: 2) same method of ascertainment for cases and controls; E3: 3) non-response rate. Goetzl-2 et al., 2015 refers to the reference of Goetzl et al., 2015b; Goetzl-4 et al., 2016 refers to the reference of Goetzl et al., 2016b; Goetzl-5 et al., 2016 refers to the reference of Goetzl et al., 2016a; Goetzl-7 et al., 2018 refers to the reference of Goetzl et al., 2018a; Jia-1 et al., 2019 refers to the reference of Jia et al., 2019; Jia-2 et al., 2020 refers to the reference of Jia et al., 2020a; Li et al., 2020 refers to the reference of Li et al., 2020b; Winston-1 et al., 2016 refers to the reference of Winston et al., 2016; Winston-2 et al., 2018 refers to the reference of Winston et al., 2018; Winston-3 et al., 2019 refers to the reference of Winston et al., 2019.

healthy control subjects (Goetzl et al., 2016a). Moreover, the blood NDE APP, soluble APPα (sAPPα), and sAPPβ levels were increased in AD patients compared with those in healthy control subjects, but the blood NDE BACE-1 and γ-secretase levels showed no significant difference (Goetzl et al., 2016a).

Blood exosomal phosphorylated and T-tau in patients with AD, MCI, or preclinical AD

We conducted a meta-analysis of biomarkers related to neurofibrillary tangles (NFTs) including blood NDE P-T181-tau, P-S396-tau, and T-tau. First, we combined seven studies (Fiandaca et al., 2015; Goetzl et al., 2016a; Winston et al., 2016; Jia et al., 2019; Gu et al., 2020; Li et al., 2020a; Nam et al., 2020) to compare the blood NDE P-T181-tau level in 271 AD patients and 258 healthy controls and found that the blood NDE P-T181-tau level in AD patients was significantly higher than that in healthy controls (SMD = 3.19, 95% CI: 1.93–4.46, $P < 0.001$; **Figure 3A**). Similarly, in four trails (Winston et al., 2016; Jia et al., 2019; Li et al., 2020a; Nam et al., 2020), the blood NDE P-T181-tau level was significantly higher in 183 patients with MCI than it was in 152 healthy controls (SMD = 3.15, 95% CI: 1.29–5.01, $P < 0.001$; **Figure 3B**). In five studies (Fiandaca et al., 2015; Winston et al., 2016; Jia et al., 2019; Li et al., 2020a; Nam et al., 2020), the blood NDE P-T181-tau level in 175 patients with AD was significantly higher compared with that in 212 MCI patients (SMD = 1.19, 95% CI: 0.09–2.30, $P < 0.05$; **Figure 3C**). Perrotte et al. (2020) did not report specific data but also showed that the blood NDE P-T181-tau level was increased in AD patients.

Second, we performed a meta-analysis of five studies (Fiandaca et al., 2015; Goetzl et al., 2016a; Winston et al., 2016; Gu et al., 2020; Li et al., 2020a) to compare the blood NDE P-S396-tau level between 152 AD patients and 134 healthy controls. We found that the blood NDE P-S396-tau level in AD patients was significantly higher than that in healthy controls (SMD = 4.58, 95% CI: 2.41–6.75, $P < 0.001$; **Figure 4A**). Similar results were not observed when we combined three studies (Winston et al., 2016, 2018; Li et al., 2020a) to analyze the blood NDE P-S396-tau level in 99 patients with MCI and 104 healthy subjects (SMD = 1.61, 95% CI: –0.18–3.39, $P = 0.08$; **Figure 4B**). Moreover, the blood NDE P-S396-tau level was compared between 56 AD patients and 67 MCI patients extracted from three studies (Fiandaca et al., 2015; Winston et al., 2016; Li et al., 2020a). The results showed that the blood NDE P-S396-tau level in AD patients was significantly increased compared with that in MCI patients (SMD = 4.27, 95% CI: 1.06–7.48, $P < 0.01$; **Figure 4C**). Additionally, Sun et al. (2020) showed without specific data that P-S396-tau in AD patients was significantly higher than that in healthy control subjects.

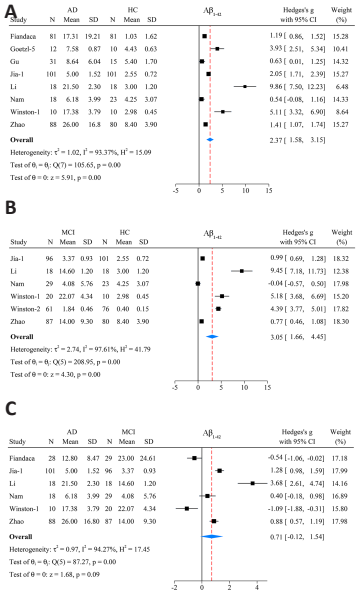

Figure 2 | **Results of meta-analysis regarding blood exosomal $A\beta_{1-42}$ levels in AD and MCI patients and in healthy control (HC) subjects.**
The SMD and corresponding 95% CIs of the $A\beta_{1-42}$ level between AD patients and HC subjects are shown in a forest plot. (B) The SMD and corresponding 95% CIs of the $A\beta_{1-42}$ level between MCI patients and HC subjects are shown in a forest plot. (C) The SMD and corresponding 95% CIs of the $A\beta_{1-42}$ level between AD and MCI patients are shown in a forest plot. The sign after the author is to distinguish studies of authors with the same last name or studies with the same first author, and the number represents the order of the article. AD: Alzheimer's disease; CI: confidence interval; HC: healthy control; MCI: mild cognitive impairment; SMD: standard mean difference. Note: Goetzl-5 refers to the reference of Goetzl et al., 2016a; Jia-1 refers to the reference of Jia et al., 2019; Li refers to the reference of Li et al., 2020b; Winston-1 refers to the reference of Winston et al., 2016; Winston-2 refers to the reference of Winston et al., 2018.

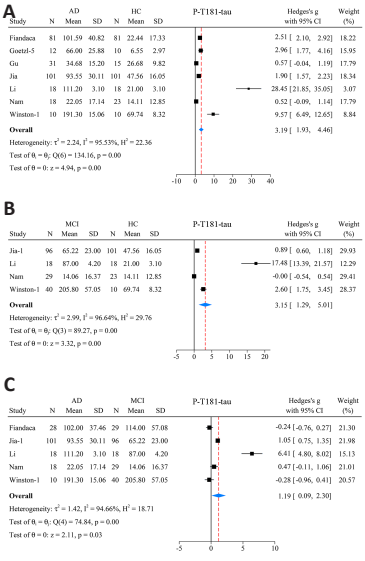

Figure 3 | **Results of meta-analysis regarding blood exosomal P-T181-tau levels in AD and MCI patients and HC subjects.**
The SMD and corresponding 95% CIs of the P-T181-tau level between AD patients and HC subjects are shown in a forest plot. (B) The SMD and corresponding 95% CIs of the P-T181-tau level in MCI patients and HC subjects are shown in a forest plot. (C) The SMD and corresponding 95% CIs of the P-T181-tau level in AD and MCI patients are shown in a forest plot. The sign after the author is to distinguish studies of authors with the same last name or studies with the same first author, and the number represents the order of the article. AD: Alzheimer's disease; CI: confidence interval; HC: healthy control; MCI: mild cognitive impairment; P-T181-tau: phosphorylated tau (threonine 181); SMD: standard mean difference. Note: Goetzl-5 refers to the reference of Goetzl et al., 2016a; Jia-1 refers to the reference of Jia et al., 2019; Li refers to the reference of Li et al., 2020b; Winston-1 refers to the reference of Winston et al., 2016.

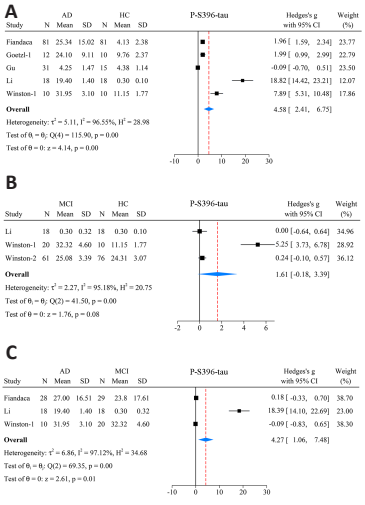

Figure 4 | **Results of meta-analysis regarding blood exosomal P-S396-tau levels in AD and MCI patients and HC subjects.**
(A) The SMD and corresponding 95% CIs of the P-S396-tau level between AD patients and HC subjects are shown in a forest plot. (B) The SMD and corresponding 95% CIs of the P-S396-tau level between MCI patients and HC subjects are shown in a forest plot. (C) The SMD and corresponding 95% CIs of the P-S396-tau level between patients with AD and MCI are shown in a forest plot. The sign after the author is to distinguish studies of authors with the same last name or studies with the same first author, and the number represents the order of the article. AD: Alzheimer's disease; CI: confidence interval; HC: healthy control; MCI: mild cognitive impairment; P-S396-tau: phosphorylated tau (serine 396); SMD: standard mean difference. Note: Goetzl-1 refers to the reference of Goetzl et al., 2018b; Li refers to the reference of Li et al., 2020b; Winston-1 refers to the reference of Winston et al., 2016; Winston-2 refers to the reference of Winston et al., 2018.

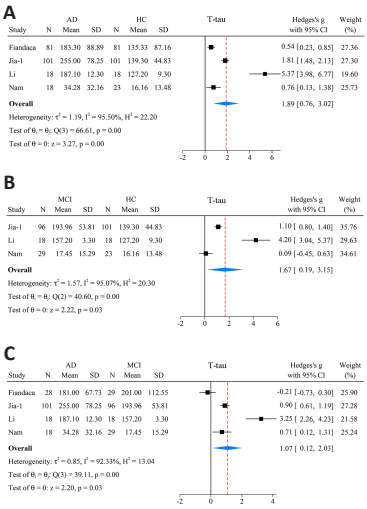

Figure 5 | **Results of meta-analysis regarding blood exosomal T-tau levels in AD and MCI patients and HC subjects.**
(A) The SMD and corresponding 95% CIs of the T-tau level between AD patients and HC subjects are shown in a forest plot. (B) The SMD and corresponding 95% CIs of the T-tau level between patients with MCI and HC subjects are shown in a forest plot. (C) The SMD and corresponding 95% CIs of the T-tau level between patients with AD and MCI are shown in a forest plot. The sign after the author is to distinguish studies of authors with the same last name or studies with the same first author, and the number represents the order of the article. AD: Alzheimer's disease; CI: confidence interval; HC: healthy control; MCI: mild cognitive impairment; SMD: standard mean difference; T-tau: total tau. Note: Jia-1 refers to the reference of Jia et al., 2019; Li refers to the reference of Li et al., 2020b.

Third, we performed meta-analysis to analyze the blood NDE T-tau level in 218 AD patients and 223 healthy controls based on four studies (Fiandaca et al., 2015; Jia et al., 2019; Li et al., 2020a; Nam et al., 2020), which showed that NDE T-tau in AD patients was significantly higher than that in healthy control subjects (SMD = 1.89, 95% CI: 0.76–3.02, $P < 0.01$; **Figure 5A**). Furthermore, compared with 142 healthy subjects, the blood NDE T-tau level was significantly higher in 143 MCI patients (SMD = 1.67, 95% CI: 0.19–3.15, $P < 0.05$; **Figure 5B**) (Jia et al., 2019; Li et al., 2020a; Nam et al., 2020), whereas it was significantly lower than that in AD patients (SMD = 1.07, 95% CI: 0.12–2.03, $P < 0.05$; **Figure 5C**) (Fiandaca et al., 2015; Jia et al., 2019; Li et al., 2020a; Nam et al., 2020). Additionally, Perrotte et al. (2020) demonstrated that the blood NDE T-tau level was increased in patients with MCI. Moreover, the blood NDE P-T181-tau and P-S396-tau levels in preclinical AD and AD patients were significantly higher than those in healthy subjects, but there was no difference in the blood NDE T-tau level between preclinical AD patients and healthy subjects (Fiandaca et al., 2015). Furthermore, the blood NDE P-S202-tau level was significantly higher in AD patients than in MCI patients and healthy controls (Nam et al., 2020).

Blood exosome-based biomarkers associated with synaptic function in patients with AD, MCI, or preclinical AD

For postsynaptic neurogranin, meta-analysis performed based on four studies (Goetzl et al., 2016b; Winston et al., 2016, 2018; Jia et al., 2020a) found that the blood NDE neurogranin level in 132 AD patients was significantly lower than that in 132 healthy subjects (SMD = –5.66, 95% CI: –9.11 to –2.21, $P < 0.01$; **Figure 6A**) (Goetzl et al., 2016b; Winston et al., 2016; Jia et al., 2020a). Similarly, compared with 187 healthy controls, the blood NDE neurogranin level was significantly lower in 197 MCI patients (SMD = –2.77, 95% CI: –5.20 to –0.34, $P < 0.05$; **Figure 6B**) (Winston et al., 2016, 2018; Jia et al., 2020a). When comparing 111 AD patients with 136 MCI patients (Winston et al., 2016; Jia et al., 2020a), a random effects model showed that the blood NDE neurogranin level in the AD patients was slightly, but not significantly, lower than that in the MCI patients (SMD = –2.78, 95% CI: –6.04–0.48, $P = 0.09$; **Figure 6C**).

Next, we compared the level of blood NDE growth-associated protein 43 (GAP43, also called neuromodulin) in 122 AD patients, 157 MCI patients, and 198 healthy control subjects from three studies (Goetzl et al., 2016b; Winston et al., 2018; Jia et al., 2020a). The fixed effects model results showed that the blood NDE GAP43 level in AD patients was significantly lower than that in healthy control subjects (SMD = –1.37, 95% CI: –1.64 to –1.09, $P < 0.01$; **Additional Figure 1A**) (Goetzl et al., 2016b; Jia et al., 2020a), and that in MCI patients it was also significantly decreased compared with healthy control subjects (SMD = –0.96, 95% CI: –1.64 to –0.27, $P < 0.01$; **Additional Figure 1B**) (Winston et al., 2018; Jia et al., 2020a).

We also compared the blood NDE synaptosomal-associated protein-25 (SNAP-25) level in 125 AD patients, 96 MCI patients, and 118 healthy control subjects from two studies (Agliardi et al., 2019; Jia et al., 2020a). The fixed effects model results showed that the blood NDE SNAP-25 level in the AD patients was significantly lower than that in the healthy control subjects (SMD = –1.01,

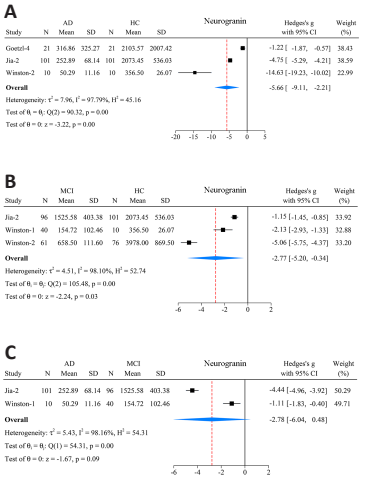

Figure 6 | **Results of meta-analysis of blood exosomal neurogranin levels in AD and MCI patients and HC subjects.**
The SMD and corresponding 95% CIs of the neurogranin level between AD patients and HC subjects are shown in a forest plot. (B) The SMD and corresponding 95% CIs of the neurogranin level between MCI patients and HC subjects are shown in a forest plot. (C) The SMD and corresponding 95% CIs of the neurogranin level between patients with AD and MCI are shown in a forest plot. The sign after the author is to distinguish authors with the same last name or studies with the same first author, and the number represents the order of the article. AD: Alzheimer's disease; CI: confidence interval; HC: healthy control; MCI: mild cognitive impairment; SMD: standard mean difference. Note: Goetzl-4 refers to the reference of Goetzl et al., 2016b; Jia-2 refers to the reference of Jia et al., 2020a; Winston-1 refers to the reference of Winston et al., 2016; Winston-2 refers to the reference of Winston et al., 2018.

95% CI: –1.28 to –0.74, $P < 0.001$; **Additional Figure 1C**) (Agliardi et al., 2019; Jia et al., 2020a).

Furthermore, compared with healthy control subjects, the blood NDE synaptotagmin, synaptopodin, and synaptophysin levels were significantly lower in AD patients (Agliardi et al., 2019; Jia et al., 2020a). Additionally, the levels of the blood NDE presynaptic membrane proteins neuronal pentraxin 2 (NPTX2) and presynaptic neurexin2 (NRXN2) and their corresponding postsynaptic membrane receptors glua4-containing glutamate (AMPA4) and neuroligin1 were significantly decreased in AD patients compared with healthy controls (Goetzl et al., 2018b). Similarly, the blood NDE synaptotagmin, synaptopodin, and synaptophysin levels were significantly decreased in MCI patients compared with healthy controls (Goetzl et al., 2016b; Jia et al., 2020a).

Three studies reported a significant decrease in the levels of the blood NDE synaptotagmin, synaptopodin, synaptophysin, neurogranin, GAP43, SNAP-25, AMPA4, neuroligin1, and NRXN2 in preclinical AD patients, compared with healthy control subjects, and the levels of these blood NDE synaptic proteins were further decreased as the patients progressed to the AD stage (Goetzl et al., 2016b, 2018b; Jia et al., 2020a).

Blood exosome-based biomarkers associated with inflammation in patients with AD or preclinical AD

The complement system is a key component of innate immunity that responds to the inflammatory process (Lee et al., 2019; Tenner, 2020). We identified two studies, including 64 AD patients and 64 healthy controls, that compared the level of complement effector proteins and complement regulatory proteins in blood ADE between patients with AD and preclinical AD (Goetzl et al., 2018a; Winston et al., 2019).

First, we compared the level of five complement effector proteins in blood ADE, including complement 1q (C1q), C4b, complement factor B-derived fragment Bb, C3b, and C5b-C9 terminal complement complex (C5b-C9 TCC). The results showed that compared with healthy controls, the levels of blood ADE C1q, C4b, factor B-derived fragment Bb, C3b, and C5b-C9 TCC in AD patients were significantly increased (C1q: SMD = 3.16, 95% CI: 2.57–3.76, $P < 0.001$; C4b: SMD = 3.38, 95% CI: 2.84–3.92, $P < 0.001$; factor B-derived fragment Bb: SMD = 2.61, 95% CI: 1.63–3.58, $P < 0.001$; C3b: SMD = 2.88, 95% CI: 2.39–3.38, $P < 0.001$; C5b-C9 TCC: SMD = 1.80, 95% CI: 1.39–2.20, $P < 0.001$; **Additional Figure 2A–E**).

Second, we compared the level of five complement regulatory proteins in blood ADE, including CD59, CD46, complement receptor 1 (CR1), decay-accelerating factor (DAF, also called CD55), and mannose-binding lectin (MBL). The results showed that the levels of blood ADE CD59, CD46, DAF, and CR1 in AD patients were significantly lower than those in healthy controls (CD59: SMD = –3.18, 95% CI: –3.70 to –2.66, $P < 0.001$; CD46: SMD = –1.20, 95% CI: –1.63 to –0.78, $P < 0.001$; DAF: SMD =–2.48, 95% CI: –2.94 to –2.03, $P < 0.001$; CR1: SMD =-0.95, 95% CI: –1.37 to –0.54, $P < 0.001$; **Additional Figure 3A–D**) and the blood ADE MBL level in AD patients was slightly, but not significantly, lower than that in healthy controls (SMD = 0.28, 95% CI: –0.11–0.68, $P = 0.16$; **Additional Figure 3E**).

Moreover, the levels of the above-mentioned five complement effector proteins in blood ADE of patients with preclinical AD were significantly lower than those in AD patients, but there was no significant difference compared with healthy controls (Goetzl et al., 2018a). However, for the complement regulatory proteins, the levels of blood ADE CD59 and DAF in preclinical AD patients were significantly lower than those in healthy controls (Goetzl et al., 2018a). This study also demonstrated that the levels of interleukin (IL)-6, tumor necrosis factor-α, and IL-1β in blood ADE were significantly higher in AD patients than those in age- and gender-matched controls (Goetzl et al., 2018a).

The main function of matrix metalloproteinases (MMPs) is to degrade and remodel the dynamic balance of the extracellular matrix. It has been reported that the MMP-9 level in plasma NDE was significantly higher in patients with AD than that in control subjects (Wang et al., 2020). Aharon et al. (2020) reported increased levels of inflammation-related proteins and cytokines, e.g., interferon-gamma, chemokine (C-C motif) ligand 5 (CCL5), C-X-C motif chemokine ligand 1 (CXCL1), IL-2, and IL-8, in blood EVs of AD patients compared with healthy controls.

Blood exosome-based biomarkers associated with metabolism in patients with AD or preclinical AD

Two studies reported the changes in the insulin receptor substrate (IRS) levels in blood NDE, including total IRS-1 (T-IRS-1) and the phosphorylated forms P-serine 312-IRS-1 (P-S312-IRS-1) and P-pan-tyrosine-IRS-1 (P-panY-IRS-1) (Kapogiannis et al., 2015, 2019). The results showed that compared with healthy controls, the blood NDE P-S312-IRS-1 and P-panY-IRS-1 levels were significantly increased and decreased, respectively, in preclinical AD and AD patients, but T-IRS-1 remained unchanged (Kapogiannis et al., 2015), but there were no statistical differences in the blood NDE P-S312-IRS-1 and P-panY-IRS-1 levels between preclinical AD and AD patients (Kapogiannis et al., 2015). Conversely, Kapogiannis et al. (2019) showed different results, namely, the blood NDE P-S312-IRS-1 and P-panY-IRS-1 levels in AD patients were significantly higher than those in healthy control subjects. Additionally, it has been reported that compared with healthy controls, the level of agouti-related peptide in AD patients was higher, whereas the N-(1-carboxymethyl)-L-lysine (CML) level was lower (Haddad et al., 2019; Aharon et al., 2020).

Blood exosome-based biomarkers associated with neurotrophy and neural growth in AD patients

Goetzl et al. (2016a) reported that the level of blood ADE glial-derived neurotrophic factor in AD patients was significantly lower than that in healthy control subjects, but no statistical difference was found in blood NDE. Furthermore, Goetzl et al. (2019) showed that the levels of five growth factors, including hepatocyte growth factor (HGF), fibroblast growth factor (FGF)-2, FGF-13, FGF-4, and type 1 insulin-like growth factor (IGF-1), in blood exosomes derived from chondroitin sulfate proteoglycan 4 type neural precursor cells (CSPG4Es) of AD patients were significantly lower than those in healthy controls.

Blood exosome-based biomarkers associated with vascular function in AD patients

Aharon et al. (2020) reported that the levels of vascular endothelial growth factor (VEGF) D and its receptors VEGFR-2 and VEGFR-3, and angiopoietin 1 in EVs were decreased, whereas the levels of platelet-derived growth factor BB and thrombopoietin were increased in AD patients compared with healthy controls.

Blood exosome-based biomarkers associated with the autophagy-lysosome system in patients with AD or preclinical AD

The autophagy-lysosome system is an important degradation pathway for clearing abnormal protein accumulation in animal models of AD (Kerr et al., 2017; Xin et al., 2018). To date, Goetzl et al. (2015a) reported that the levels of autophagy-lysosome-related proteins including cathepsin D, lysosome-associated membrane protein 1 (LAMP-1), and ubiquitinylated proteins in blood NDE of preclinical AD and AD patients were significantly increased, whereas heat shock protein 70 (HSP70) was significantly decreased, compared with healthy controls.

Blood exosomal transcription-related factors in AD patients

Two studies, including 50 AD patients and 46 healthy controls, were included in the meta-analysis. These studies showed that the blood NDE repressor element 1-silencing transcription (REST) level in AD patients was slightly, but not significantly, lower than that in healthy controls (SMD = –4.14, 95% CI: –9.75–1.46, $P = 0.15$; **Additional Figure 4**). The levels of low-density lipoprotein receptor-related protein 6 (LRP6) and heat-shock factor 1 (HSF1) significantly decreased in blood NDE of preclinical AD and AD patients, compared with healthy control subjects (Goetzl et al., 2015b). The level of blood NDE TAR DNA-binding protein of 43 kDa (TDP-43), a crucial transcription factor with a fundamental role in metabolic processing of tau mRNA, was elevated in AD patients compared with healthy controls (Zhang et al., 2020).

Blood exosomal miRNAs in patients with AD or MCI

Nine studies reported that miRNA derived from blood exosomes may be used as biomarkers for the diagnosis of AD (Cheng et al., 2015; Lugli et al., 2015; Yang et al., 2018; Cha et al., 2019; Gamez-Valero et al., 2019; Aharon et al., 2020; Li et al., 2020a; Nie et al., 2020; Serpente et al., 2020). The levels of blood NDE miR-212 and miR-132 in AD patients were significantly lower than those in healthy control subjects (Cha et al., 2019). Serum exosomal miR-135a, miR-193b, and miR-384 levels were significantly increased in AD patients compared with healthy controls (Yang et al., 2018). In contrast, plasma EV miR-23a-3p, miR-126-3p, let-7i-5p, and miR-151a-3p levels were significantly decreased in AD patients compared with healthy controls (Gamez-Valero et al., 2019). In six studies (Cheng et al., 2015; Lugli et al., 2015; Aharon et al., 2020; Li et al., 2020a; Nie et al., 2020; Serpente et al., 2020), a large number of differentially expressed miRNAs were screened by gene sequencing and microarray, of which 18 miRNAs were downregulated (including miR-23b-3p, miR-24-3p, miR-29b-3p, miR-125b-5p, miR-139-5p, miR-141-3p, miR-150-5p, miR-152-3p, miR-185-5p, miR-338-3p, miR-342-3p, miR-342-5p, miR-548a-5p, miR-3613-3p, miR-3916, miR-4772-3p, miR-1306-5p, and miR-15b-3p), whereas 17 miRNAs were upregulated (including miR-138-5p, miR-659-5p, miR-5001-3p, miR-361-5p, miR-30e-5p, miR-93-5p, miR-15a-5p, miR-143-3p, miR-335-5p, miR-106b-5p, miR-101-3p, miR-424-5p, miR-106a-5p, miR-18b-5p, miR-3065-5p, miR-20a-5p, and miR-582-5p) in AD patients compared with the healthy controls. Furthermore, another study found that the level of miR-15b-3p decreased, whereas the levels of miR-424-5p, miR-3065-5p, and miR-93-5p increased in MCI patients compared with healthy controls (Li et al., 2020a). In plasma NDE, two studies identified eight miRNAs including miR-204-5p, miR-125a-5p, miR-1468-5p, miR-375, let-7e-5p, miR-23a-3p, miR-223-3p, and miR-190a-5p that were decreased and four miRNAs including miR-423-5p, miR-369-5p, miR-23a-3p, and miR-100-3p that were increased in AD patients compared with healthy controls (Nie et al., 2020; Serpente et al., 2020).

Sensitivity analysis

Because of the high heterogeneity, we conducted a sensitivity analysis on the combined results from more than two studies (**Additional Figures 5–9**). The figures showed that a study may excessively affect the results of the meta-analysis, which may be considered as the source of heterogeneity (Li et al., 2020a). Specifically, the current inconsistency between the extraction of exosomes and detection procedures of exosome cargos is one of the main limitations of exosome-related studies. Namely, some studies used specific markers to isolate NDEs, while some studies simply extracted EVs. Additionally, the use of kits introduced the problem of low purity.

Discussion

A summary of evidence

Recently, Aging-Alzheimer's Association workgroups created a research framework based on biomarkers associated with Aβ, tau, and neurodegeneration (A/T/N) and other available biomarkers for the diagnosis of preclinical and clinical AD. It is generally believed that individuals positive for Aβ will be included in the Alzheimer's continuum. In nonspecific neurodegeneration/neuronal damage, specific pathological tau biomarkers can be isolated to further refine the diagnosis and staging criteria of AD (Jack et al., 2018). Importantly, blood exosome-based biomarkers are emerging as a novel noninvasive tool for the diagnosis of preclinical and clinical AD. Here, we conducted a comprehensive meta-analysis and systematic review of blood exosomes and found that several core molecules ($Aβ_{1–42}$, P-T181-tau,

P-S396-tau, and T-tau) and additional factors (miRNA and molecules related to neuroinflammation, metabolism disorder, neurotrophic deficiency, vascular injury, autophagy-lysosomal system dysfunction, and synaptic dysfunction) in blood exosomes can serve as candidate diagnostic biomarkers for preclinical and clinical AD.

Blood exosome-based biomarkers reflected amyloid deposition and neurofibrillary tangles in preclinical and clinical AD

In the past few decades, the core and typical pathological features of AD, i.e., amyloid deposition and NFTs, have been among the research hotspots. In histopathology, amyloid deposition is derived from the abnormal cleavage of APP, which is cleaved into N-terminal sAPPα and sAPPβ and C-terminal $Aβ_{39–43}$ peptides through three types of proteases, including α-secretase, β-secretase, and γ-secretase, and thus APP causes excessive accumulation of $Aβ_{1–42}$ peptide (Gouras et al., 2015). NFTs are made of paired helical filaments, which comprise pathological filamentous aggregations of abnormally phosphorylated tau protein (Iqbal et al., 2016).

Blood exosomes can effectively cross the blood-brain barrier and exosomes derived from nerve cells and glial cells can be secreted into the peripheral blood, which mediates intercellular information exchange and better reflects the pathological changes in nervous system diseases (Wood et al., 2011; Kalluri and LeBleu, 2020). In our study, the core pathological biomarkers reflecting amyloid deposition and NFTs ($Aβ_{1–42}$, P-T181-tau, P-S396-tau, and T-tau) were increased in blood NDE of patients with preclinical AD, MCI, or AD. Furthermore, blood NDE APP, sAPPα, and sAPPβ were increased in AD patients, but BACE-1 and γ-secretase were unchanged. Simultaneously, increases in cathepsin D, LAMP-1, and ubiquitinylated proteins, and a decrease in HSP70 in blood NDE of preclinical AD and AD patients impaired autophagy-lysosomal function, resulting in reduced clearance of abnormal Aβ and tau proteins. Moreover, the levels of $Aβ_{1–42}$, P-T181-tau, P-S396-tau, and T-tau in blood NDE of AD and MCI patients were increased, which is consistent with the levels in the CSF (Jia et al., 2019).

Jia et al. (2019) plotted a receiver operating characteristic (ROC) curve and found that the combined use of blood exosomes and CSF biomarkers improved the diagnostic ability. The process of abnormal cleavage of APP by secretase occurs in the early endosomes. The protein fragments produced bind to the exosomes, and then are secreted to the extracellular space to form amyloid deposits. Similarly, the accumulation and secretion of tau are affected by exosomes (Asai et al., 2015; Budnik et al., 2016). Namely, compared with healthy controls, exosomes can absorb more Aβ and tau in the brain of patients with AD and release them into the peripheral blood, resulting in higher levels of Aβ and tau in blood NDE. Independent studies have shown that significant increases in blood NDE Aβ and tau were detected in high-risk but cognitively normal people 10 years before the diagnosis of AD, and a further increase decrease in $Aβ_{1–42}$ was observed when AD was diagnosed (Fiandaca et al., 2015). These findings are consistent with the results of AD animal models. At present, transgenic mice that overexpress APP are commonly used to study the pathology of AD, because studies have found that Aβ deposition is the core pathological change in AD that can be detected in the early stage of AD in animal models (Sasaguri et al., 2017). As another important pathological change, tau pathology follows Aβ plaque deposition, and can be produced in an Aβ-dependent or Aβ-independent manner (van der Kant et al., 2020). It has been demonstrated that high levels of Aβ and P-tau correlate with cognitive decline in AD mouse models (Huber et al., 2018). Therefore, they may be biomarkers for predicting AD and monitoring disease progression.

Moreover, recent studies have shown that the proportion of $Aβ_{1–42}/Aβ_{1–40}$ rather than the total Aβ level plays a direct role in inducing NFTs in human neurons, thereby providing a theoretical basis for the joint detection of $Aβ_{1–42}$ and $Aβ_{1–40}$ (Kwak et al., 2020). In terms of another core biomarker, it has been reported that P-T217-tau and P-T217-tau/T-tau have a stronger correlation with the tau positron emission tomography tracer [18F]flortaucipir than P-T181-tau, thus in diagnosis, CSF P-T217-tau is better than P-T181-tau for distinguishing AD from non-AD patients, therefore P-T217-tau is expected to replace P-T181-tau as a more accurate AD biomarker (Janelidze et al., 2020).

Blood exosome-based biomarkers related to additional risk factors in preclinical and clinical AD

With the advances in AD research, it has been found that in addition to the core pathological factors, other risk factors such as neuroinflammation, metabolism disorders, neurotrophic deficiency, vascular injury, and autophagy-lysosomal system dysfunction are closely related to the aggravation of the pathological development of AD (**Figure 7**). Complement effector proteins are serum proteins that mediate immune responses and inflammatory responses, which can be activated in many ways, while complement regulatory proteins are molecules involved in the activation and regulation of the complement system in a specific way that maintains complement activation and inhibition in a fine balance (Morgan and Harris, 2015; Hajishengallis et al., 2017). The levels of ADE complement effector proteins (C1q, C4b, factor B fragment Bb, C3b, and C5b-C9 TCC) except for MBL in AD patients were significantly higher, while the levels of complement regulatory proteins (CD59, CD46, CR1, and DAF) were significantly lower, compared with healthy controls. In AD patients, the imbalance between these proteins may lead to excessive inflammatory responses. These substances were not considered as core pathological factors of AD, but our meta-analysis results suggest that they can be used as effective auxiliary biomarkers in combination with the traditional core biomarkers under the new A/T/N diagnostic framework.

A large AD proteomic study showed that the protein network modules related to glucose metabolism are closely related to the pathological development and cognitive impairment in AD, especially, the relationship between the metabolism of astrocytes and microglia was most obvious (Johnson et al., 2020), suggesting that metabolic function, especially glucose metabolism, may play an important role in the AD pathological development, and thus will be the focus of future research. Interestingly, the results of the IRS studies were not consistent. One study showed that in patients with AD, the level of P-S312-IRS-1, an insulin signal-diminishing factor, in blood NDE increased, the insulin signal-enhancing P-panY-IRS-1 decreased, and the insulin resistance factor significantly increased, and that insulin resistance led to decreased glucose uptake and abnormal metabolism in the brain (Kapogiannis et al., 2015). Another study observed significant changes in these proteins 1–10 years before the diagnosis of AD (Kapogiannis et al., 2015). However, it has been shown in another study that the levels of P-S312-IRS-1 and P-panY-IRS-1 were significantly increased, and thus can be used as important individual predictors in the establishment of an AD prediction model (Kapogiannis et al., 2019).

Animal experiments showed increased IGF-1R surrounding and within Aβ-containing plaques in the cortex, which was also seen in astrocytes in AD (Moloney et al., 2010). Another study reported that P-S616-IRS-1 was increased in the cortex and dentate gyrus of APP/PS1 mice (Denver et al., 2018). However, although the total level of IGF-1R increased, the number of IGF-1R-expressing neurons in AD significantly decreased, and IGF-1R was abnormally distributed in AD neurons. Additionally, in AD neurons, the levels of IRS-1 and IRS-2 were significantly decreased, while the levels of P-S312-IRS-1 and P-S616-IRS-1 were increased, and these increased levels were closely related to the formation of NFTs, which can impair IGF-1R and the insulin receptor signal, hence neurons may be resistant to the insulin signal (Moloney et al., 2010). Further studies found that insulin in neurons containing hyperphosphorylated tau was stored as oligomers and continued to accumulate with the development of tau pathology. These neurons also showed signs of insulin resistance and decreased levels of insulin receptors, suggesting that insulin retention may be a cause of insulin resistance in tau-related diseases (Rodriguez-Rodriguez et al., 2017).

Moreover, it has been reported that P-S616-IRS-1 and P-S636/639-IRS-1 positively correlated with Aβ plaques and negatively correlated with working memory, even after adjusting other core pathological features, and that the candidate biomarkers of insulin resistance were significantly increased in patients with MCI or AD, and gradually increased during the progression of MCI to AD, suggesting that brain insulin resistance may be an early pathological feature of AD (Talbot et al., 2012). As an advanced glycation end product, the increase in CML in blood EVs is closely related to the deposition of neurotoxic proteins in the brain, oxidative stress, and neuronal loss (Byun et al., 2017), whereas in exosomes derived from blood CSPG4Es, the levels of growth factors, including HGF, FGF-2, FGF-13, and IGF-1, were significantly decreased, indicating that AD patients have a variety of cell growth defects and dysfunctions, which are not limited to nerve cells (Goetzl et al., 2019).

Additionally, the central nervous system of patients with AD has autophagy-lysosomal system dysfunction, which is manifested by an increase in the number of lysosomes along with protein biodegradation dysfunction (Ihara et al., 2012). This results in lysosome inherent proteins leaking into exosomes and binding with them to remove unnecessary proteins, such as cathepsin D, LAMP-1, and ubiquitin proteins designed to be cleared by lysosomes, which can result in an increase in the protein level in blood NDE; however, a lower level of NDE HSP70 leads to a decrease in the clearance rate of neurotoxic proteins (Schwagerl et al., 1995; Goldfarb et al., 2006; Goetzl et al., 2015a). Interestingly, another study has reported that the level of HSP70 when quantified in exosomes was higher than when soluble in the plasma, and that HSP70 in exosomes showed different trends among various stages of dementia, namely, it increases in the early stage of the disease and decreases in the moderate and advanced stages (Chanteloup et al., 2019). Additionally, the current results showed that HSP70 is not specific for distinguishing AD from frontotemporal dementia, and thus further investigation is required (Chanteloup et al., 2019).

Apart from the above studies, some studies were carried out at the gene level. The level of REST in blood NDE of AD patients was decreased, but no statistical significance was observed. As a repressor protein, REST binds to a silencer DNA sequence, hindering the translation of RNA into protein and effectively regulating neural differentiation (Lu et al., 2014). Additionally, the abnormal expression of transcription factors HSF1 and TDP-43 affects RNA splicing, transport, and translation. Furthermore, several gene sequencing and microarray studies have screened the differentially expressed miRNA in the blood exosomes of patients with AD and selected several miRNAs with larger differentially expressed multiples for a verification test and diagnostic ability test. The ROC curve showed that miR-132 and miR-212 in blood NDE had a good ability to distinguish AD from the control group. Moreover, among miR-135a, miR-193b, and miR-384, miR-384 showed the best ability to distinguish AD, vascular dementia, and Parkinson's disease with dementia, but the ROC curve showed that the combination of the three was better than using miR-384 alone in the diagnosis of early AD. Additionally, in blood EVs, hsa-miR-21-5p and hsa-miR-451a were more effective in distinguishing dementia with Lewy bodies from AD.

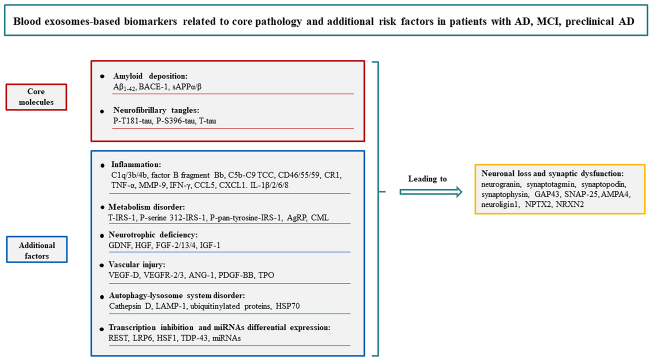

Figure 7 | Important blood exosome-based biomarkers related to core pathology and additional risk factors in patients with AD, MCI, or preclinical AD.
The green box represents the title and general frame, the red box includes the core pathological molecules, the blue box represents additional factors, and the yellow box indicates proteins related to neuronal loss and synaptic dysfunction. AD: Alzheimer's disease; AgRP: agouti-related peptide; AMPA4: GluA4-containing glutamate; ANG-1: angiopoietin 1; Aβ: β-amyloid; BACE-1: β-site amyloid precursor protein-cleaving enzyme 1; CCL5: chemokine (C-C motif) ligand 5; CML: N-(1-carboxymethyl)-L-lysine; CR1: complement receptor 1; CXCL1: C-X-C motif chemokine ligand 1; C1q: complement 1q; FGFs-2: fibroblast growth factors-2; GAP-43: growth-associated protein 43; GDNF: glial-derived neurotrophic factor; HGF: hepatocyte growth factor; HSF1: heat-shock factor 1; HSP70: heat shock protein 70; IFN-γ: interferon-gamma; IGF-1: type 1 insulin-like growth factor; IL-1β: interleukin 1β; IRS-1: insulin receptor substrate 1; LAMP-1: lysosome-associated membrane protein 1; LRP6: low-density lipoprotein receptor-related protein 6; MCI: mild cognitive impairment; MMP-9: matrix metalloproteinase-9; miR: microRNA; NPTX2: neuronal pentraxin 2; NRXN2a: neurexin 2α; PDGF-BB: platelet-derived growth factor BB; P-tau: phosphorylated tau; REST: repressor element 1-silencing transcription; sAPP: soluble amyloid precursor protein; SNAP-25: synaptosomal-associated protein-25; TCC: terminal complement complex; TDP-43: TAR DNA binding protein of 43 kDa; TNF-α: tumor necrosis factor-α; TPO: thrombopoietin; T-tau: total tau; VEGFR-2: vascular endothelial growth factor receptor 2.

Blood exosome-based biomarkers monitor neural loss and synaptic dysfunction in preclinical and clinical AD

The core and additional pathological changes in neural loss and synaptic dysfunction, and the emerging biomarkers such as neurogranin in blood NDE of AD and MCI patients were significantly lower than in healthy controls. Neurogranin is a synaptic function-related protein that affects synaptic plasticity by regulating the binding and release of Ca^{2+} (Lista and Hampel, 2017; Li et al., 2020b) and its level is closely related to Aβ, P-tau, and T-tau (De Vos et al., 2015; Janelidze et al., 2016; Wellington et al., 2016, 2018). Our results showed that the level of blood NDE neurogranin in patients with AD or MCI was higher than that in healthy controls. However, because of the large differences in its absolute concentration among different studies, the results were not statistically significant. Additionally, the synaptic dysfunction reflected by the reduced neurogranin level may be earlier than the neuronal degeneration and NFTs mirrored by the tau pathology, hence it may play an important role in the early memory decline of AD patients (Wildsmith et al., 2014; Casaletto et al., 2017). Similarly, in the brain tissue of transgenic AD mouse models, a decrease in the neurogranin level was detected. In contrast, intrahippocampal injection of a lentiviral vector expressing neurogranin upregulated the expression level of neurogranin, which improved the cognitive function and the expression level of synapse-related proteins in AD mice (Esteve et al., 2017; Jeon et al., 2018).

Moreover, in our qualitative analysis, the levels of synaptic proteins such as synaptotagmin, synaptopodin, synaptophysin, GAP43, SNAP-25, NPTX2, AMPA4, NRXN2α, and NLGN1 in blood NDE of AD patients were significantly decreased. These results were consistent with the pathology of synaptic dysfunction reflected by the abnormal changes in neurogranin.

Protective role and therapeutic potential of exosomes in AD

Although some studies have shown that exosomes may be harmful in AD, increasing evidence indicates that they also possess beneficial actions that may be of importance in the development of AD. Recent studies have found that exosomes derived from human umbilical cord mesenchymal stem cells (hucMSCs) significantly improved cognitive dysfunction in APP/PS1 transgenic mice, and promoted Aβ degradation in the cortex and hippocampus, which was achieved by upregulating the expression levels of Aβ degrading enzymes (neprilysin and insulin degrading enzyme) (Ding et al., 2018). Additionally, it has been found that injection of hucMSC-derived exosomes reduced neuroinflammation by regulating the activation of glial cells and the levels of inflammatory factors in the brain of model mice (Ding et al., 2018). Another study demonstrated that exosomes from hypoxia-preconditioned MSCs reduced Aβ deposition and improved learning and memory in APP/PS1 mice. The underlying mechanism may lie in the enhancement of synaptic function and regulation of inflammatory responses through regulation of miR-21 (Cui et al., 2018).

Accumulated evidence suggests that exosomes, especially those derived from MSCs, have good potential for the treatment of AD, and that they possess an excellent safety profile and minimal immunogenicity (Xian et al., 2019; Fan et al., 2020). Namely, exosome therapy has the advantages of stem cell therapy, without the risk of rejection and uncontrolled division, thereby avoiding inflammation and tumor formation (Reza-Zaldivar et al., 2018). Furthermore, exosomes cross the blood-brain barrier more easily owing to their nano-size characteristics, which are conducive to their surface modification and development into engineered exosomes, combining ligands with specific targets to achieve personalized treatment (Gorabi et al., 2019; Gandham et al., 2020). To our knowledge, as a disease therapeutic vehicle, curcumin-containing exosomes can inhibit the hyperphosphorylation of tau protein by activating relevant signaling pathways, thereby preventing neuronal death *in vitro* and *in vivo*, and thus providing a beneficial effect on the cognition of AD model mice (Wang et al., 2019).

However, using exosomes as a clinical treatment still faces great challenges. First, the exosome isolation and purification procedures need to be continuously optimized and standardized. In the included studies, most of them used commercial kits to extract and isolate exosomes, while five studies chose ultracentrifugation (Cheng et al., 2015; Lugli et al., 2015; Cha et al., 2019; Aharon et al., 2020; Ellegaard et al., 2020) and one study used size exclusion chromatography (Gamez-Valero et al., 2019). A polyethylene glycol precipitation method was used in most of the kits, resulting in impaired purity of the exosomes, which affected the accuracy of the results. Second, the development of effective biomarkers for exosomes requires sensitive, accurate, and rapid quantification methods. In recent years, the emergence of single molecule arrays provided broad prospects for this. However, most of the included studies chose traditional PCR and enzyme-linked immunosorbent assay to quantify exosomal cargos, and only two studies used high-sensitivity and high-throughput techniques of mesoscale discovery (Kapogiannis et al., 2019) and Luminex (Perrotte et al., 2020), which limited the application of exosomal biomarkers in disease diagnosis. Third, before exosomes can be accepted for clinical treatment, more research on the source of exosomes, the time of administration, the side effects of drugs, and the effective route of administration needs to be carried out. Finally, the current clinical trials related to exosomes were mostly observational studies. The cohorts in the included studies were mostly from the United States and China, and their sample size was small, which may lead to a racial bias of the AD biomarkers screened and prevent worldwide application. It is particularly noteworthy that although there were many clinical treatment trials that have been approved, more experimental work is needed before extensive clinical trials can be performed. Based on the promising results of preclinical studies so far, exosome-based diagnosis and therapy is a potential option and is steadily moving towards clinical applications (Guo et al., 2020a; Yin et al., 2020).

Study limitations

Our research has some limitations. Firstly, there was a large heterogeneity between the included studies and the sensitivity analysis showed that Li et al. (2020a) may have excessively affected the combined effect size of the analysis. The reason may be that this study detected the level of biomarkers in serum EVs, without specific description of whether they were from NDE. Secondly, the appearance of high heterogeneity may be because of the lack of unified procedures for the extraction and isolation of exosomes and for the detection of biomarkers. Different reagents and detection methods also cause methodological heterogeneity, leading to a significant difference in absolute substance concentrations between studies, therefore we chose SMD instead of MD as our effect size, and consequently, the accurate cut-off concentration could not be given in this study. Additionally, the geographical origins of the cohorts were mainly concentrated in certain regions, which may lead to biased results.

Applicability and implications for future research

Many of the Aβ- and tau-based treatments have failed in curing AD, raising the question of whether amyloid deposition and NFTs remain the primary neuropathology of AD. However, this study demonstrated the abnormally altered blood exosome-based biomarkers in preclinical and clinical AD, confirming the importance of some of them in the pathogenesis of preclinical and clinical AD and supporting the idea that $A\beta_{1-42}$, P-T181-tau, P-S396-tau, T-tau, neurogranin, and other molecules involved in the complex pathological mechanisms of AD in the blood exosomes can be used as biomarkers to distinguish AD, MCI, or preclinical AD from healthy subjects (**Figure 7**). These findings need to be confirmed in further longitudinal studies based on larger populations and broader geographical origins. In general, this study aimed to increase the focus on biomarkers that can specifically distinguish AD from other neurodegenerative diseases and to broaden the horizons of AD diagnosis and treatment.

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Conflicts of interest: *The authors declare that they have no conflict of interest.*
Reporting statement: This study followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement.
Availability of data and materials: *All data generated or analyzed during this study are included in this published article and its supplementary information files.*

Additonal files:

Additonal Table 1: *Characteristics of the included studies.*

Additional Figure 1: *Results of meta-analysis in blood exosomal GAP-43 and SNAP-25 levels among patients with AD, MCI and HCs.*

Additional Figure 2: *Results of meta-analysis in levels of blood exosomal complement effector proteins between patients with AD and HCs.*

Additional Figure 3: *Results of meta-analysis in levels of blood exosomal complement regulatory proteins between patients with AD and HCs.*

Additional Figure 4: *Results of meta-analysis in blood exosomal REST level between patients with AD and HCs.*

Additional Figure 5: *Results of sensitivity analysis in blood exosomal $A\beta_{1-42}$ levels among patients with AD, MCI and HCs.*

Additional Figure 6: *Results of sensitivity analysis in blood exosomal P-T181-tau levels among patients with AD, MCI and HCs.*

Additional Figure 7: *Results of sensitivity analysis in blood exosomal P-S396-tau levels among patients with AD, MCI and HCs.*

Additional Figure 8: *Results of sensitivity analysis in blood exosomal T-tau levels among patients with AD, MCI and HCs.*

Additional Figure 9: *Results of sensitivity analysis in blood exosomal neurogranin levels among patients with AD, MCI and HCs.*

References

Agliardi C, Guerini FR, Zanzottera M, Bianchi A, Nemni R, Clerici M (2019) SNAP-25 in serum is carried by exosomes of neuronal origin and is a potential biomarker of alzheimer's disease. Mol Neurobiol 56:5792-5798.

Aharon A, Spector P, Ahmad RS, Horrany N, Sabbach A, Brenner B, Aharon-Peretz J (2020) Extracellular vesicles of alzheimer's disease patients as a biomarker for disease progression. Mol Neurobiol 57:4156-4169.

Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7:270-279.

Asai H, Ikezu S, Tsunoda S, Medalla M, Luebke J, Haydar T, Wolozin B, Butovsky O, Kugler S, Ikezu T (2015) Depletion of microglia and inhibition of exosome synthesis halt tau propagation. Nat Neurosci 18:1584-1593.

Budnik V, Ruiz-Canada C, Wendler F (2016) Extracellular vesicles round off communication in the nervous system. Nat Rev Neurosci 17:160-172.

Byun K, Yoo Y, Son M, Lee J, Jeong GB, Park YM, Salekdeh GH, Lee B (2017) Advanced glycation end-products produced systemically and by macrophages: a common contributor to inflammation and degenerative diseases. Pharmacol Ther 177:44-55.

Casaletto KB, Elahi FM, Bettcher BM, Neuhaus J, Bendlin BB, Asthana S, Johnson SC, Yaffe K, Carlsson C, Blennow K, Zetterberg H, Kramer JH (2017) Neurogranin, a synaptic protein, is associated with memory independent of Alzheimer biomarkers. J Neurol 89:1782-1788.

Cha DJ, Mengel D, Mustapic M, Liu W, Selkoe DJ, Kapogiannis D, Galasko D, Rissman RA, Bennett DA, Walsh DM (2019) miR-212 and miR-132 are downregulated in neurally derived plasma exosomes of Alzheimer's patients. Front Neurosci 13:1208.

Chanteloup G, Cordonnier M, Moreno-Ramos T, Pytel V, Matías-Guiu J, Gobbo J, Cabrera-Martín MN, Gómez-Pinedo U, Garrido C, Matías-Guiu JA (2019) Exosomal HSP70 for monitoring of frontotemporal dementia and Alzheimer's disease: clinical and FDG-PET correlation. Alzheimers Dis 71:1263-1269.

Cheng L, Doecke JD, Sharples RA, Villemagne VL, Fowler CJ, Rembach A, Martins RN, Rowe CC, Macaulay SL, Masters CL, Hill AF (2015) Prognostic serum miRNA biomarkers associated with Alzheimer's disease shows concordance with neuropsychological and neuroimaging assessment. Mol Psychiatry 20:1188-1196.

Cui GH, Wu J, Mou FF, Xie WH, Wang FB, Wang QL, Fang J, Xu YW, Dong YR, Liu JR, Guo HD (2018) Exosomes derived from hypoxia-preconditioned mesenchymal stromal cells ameliorate cognitive decline by rescuing synaptic dysfunction and regulating inflammatory responses in APP/PS1 mice. FASEB J 32:654-668.

Cumpston M, Li T, Page MJ, Chandler J, Welch VA, Higgins JP, Thomas J (2019) Updated guidance for trusted systematic reviews: a new edition of the Cochrane Handbook for Systematic Reviews of Interventions. Cochrane Database Syst Rev 10:Ed000142.

De Vos A, Jacobs D, Struyfs H, Fransen E, Andersson K, Portelius E, Andreasson U, De Surgeloose D, Hernalsteen D, Sleegers K, Robberecht C, Van Broeckhoven C, Zetterberg H, Blennow K, Engelborghs S, Vanmechelen E (2015) C-terminal neurogranin is increased in cerebrospinal fluid but unchanged in plasma in Alzheimer's disease. Alzheimers Dement 11:1461-1469.

Denver P, English A, McClean PL (2018) Inflammation, insulin signaling and cognitive function in aged APP/PS1 mice. Brain Behav Immun 70:423-434.

DerSimonian R, Laird N (2015) Meta-analysis in clinical trials revisited. Contemp Clin Trials 45:139-145.

Ding M, Shen Y, Wang P, Xie Z, Xu S, Zhu Z, Wang Y, Lyu Y, Wang D, Xu L, Bi J, Yang H (2018) Exosomes isolated from human umbilical cord mesenchymal stem cells alleviate neuroinflammation and reduce amyloid-beta deposition by modulating microglial activation in Alzheimer's disease. Neurochem Res 43:2165-2177.

Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, Delacourte A, Galasko D, Gauthier S, Jicha G, Meguro K, O'Brien J, Pasquier F, Robert P, Rossor M, Salloway S, Stern Y, Visser PJ, Scheltens P (2007) Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol 6:734-746.

Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, DeKosky ST, Gauthier S, Selkoe D, Bateman R, Cappa S, Crutch S, Engelborghs S, Frisoni GB, Fox NC, Galasko D, Habert MO, Jicha GA, Nordberg A, Pasquier F, et al. (2014) Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol 13:614-629.

Ellegaard NJ, Sofie PK, Vestergård K, Georgiana MR, Christiansen G, Lundbye-Christensen S, Moos T, Risom KS, Pedersen S (2020) Novel blood-derived extracellular vesicle-based biomarkers in Alzheimer's disease identified by proximity extension assay. Biomedicines 8:199.

Eren E, Hunt J, Shardell M, Chawla S, Tran J, Gu J, Vogt NM, Johnson SC, Bendlin BB, Kapogiannis D (2020) Extracellular vesicle biomarkers of Alzheimer's disease associated with sub-clinical cognitive decline in late middle age. Alzheimers Dement 16:1293-1304.

Esteve C, Jones EA, Kell DB, Boutin H, McDonnell LA (2017) Mass spectrometry imaging shows major derangements in neurogranin and in purine metabolism in the triple-knockout 3×Tg Alzheimer mouse model. Biochim Biophys Acta Proteins Proteom 1865:747-754.

Fan B, Li C, Szalad A, Wang L, Pan W, Zhang R, Chopp M, Zhang ZG, Liu XS (2020) Mesenchymal stromal cell-derived exosomes ameliorate peripheral neuropathy in a mouse model of diabetes. Diabetologia 63:431-443.

Fiandaca MS, Kapogiannis D, Mapstone M, Boxer A, Eitan E, Schwartz JB, Abner EL, Petersen RC, Federoff HJ, Miller BL, Goetzl EJ (2015) Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: a case-control study. Alzheimers Dement 11:600-607.e601.

Gamez-Valero A, Campdelacreu J, Vilas D, Ispierto L, Rene R, Alvarez R, Armengol MP, Borras FE, Beyer K (2019) Exploratory study on microRNA profiles from plasma-derived extracellular vesicles in Alzheimer's disease and dementia with Lewy bodies. Transl Neurodegener 8:31.

Gandham S, Su X, Wood J, Nocera AL, Alli SC, Milane L, Zimmerman A, Amiji M, Ivanov AR (2020) Technologies and standardization in research on extracellular vesicles. Trends Biotechnol 38:1066-1098.

Goetzl EJ, Boxer A, Schwartz JB, Abner EL, Petersen RC, Miller BL, Kapogiannis D (2015a) Altered lysosomal proteins in neural-derived plasma exosomes in preclinical Alzheimer disease. Neurology 85:40-47.

Goetzl EJ, Boxer A, Schwartz JB, Abner EL, Petersen RC, Miller BL, Carlson OD, Mustapic M, Kapogiannis D (2015b) Low neural exosomal levels of cellular survival factors in Alzheimer's disease. Ann Clin Transl Neurol 2:769-773.

Goetzl EJ, Mustapic M, Kapogiannis D, Eitan E, Lobach IV, Goetzl L, Schwartz JB, Miller BL (2016a) Cargo proteins of plasma astrocyte-derived exosomes in Alzheimer's disease. FASEB J 30:3853-3859.

Goetzl EJ, Kapogiannis D, Schwartz JB, Lobach IV, Goetzl L, Abner EL, Jicha GA, Karydas AM, Boxer A, Miller BL (2016b) Decreased synaptic proteins in neuronal exosomes of frontotemporal dementia and Alzheimer's disease. FASEB J 30:4141-4148.

Goetzl EJ, Schwartz JB, Abner EL, Jicha GA, Kapogiannis D (2018a) High complement levels in astrocyte-derived exosomes of Alzheimer disease. Ann Neurol 83:544-552.

Goetzl EJ, Nogueras-Ortiz C, Mustapic M, Mullins RJ, Abner EL, Schwartz JB, Kapogiannis D (2019) Deficient neurotrophic factors of CSPG4-type neural cell exosomes in Alzheimer disease. FASEB J 33:231-238.

Goetzl EJ, Abner EL, Jicha GA, Kapogiannis D, Schwartz JB (2018b) Declining levels of functionally specialized synaptic proteins in plasma neuronal exosomes with progression of Alzheimer's disease. FASEB J 32:888-893.

Goldfarb SB, Kashlan OB, Watkins JN, Suaud L, Yan W, Kleyman TR, Rubenstein RC (2006) Differential effects of Hsc70 and Hsp70 on the intracellular trafficking and functional expression of epithelial sodium channels. Proc Natl Acad Sci U S A 103:5817-5822.

Gorabi AM, Kiaie N, Barreto GE, Read MI, Tafti HA, Sahebkar A (2019) The Therapeutic potential of mesenchymal stem cell-derived exosomes in treatment of neurodegenerative diseases. Mol Neurobiol 56:8157-8167.

Gouras GK, Olsson TT, Hansson O (2015) β-Amyloid peptides and amyloid plaques in Alzheimer's disease. Neurotherapeutics 12:3-11.

Gu D, Liu F, Meng M, Zhang L, Gordon ML, Wang Y, Cai L, Zhang N (2020) Elevated matrix metalloproteinase-9 levels in neuronal extracellular vesicles in Alzheimer's disease. Ann Clin Transl Neurol 7:1681-1691.

Guo M, Yin Z, Chen F, Lei P (2020a) Mesenchymal stem cell-derived exosome: a promising alternative in the therapy of Alzheimer's disease. Alzheimers Res Ther 12:109.

Guo T, Zhang D, Zeng Y, Huang TY, Xu H, Zhao Y (2020b) Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. Mol Neurodegener 15:40.

Haddad M, Perrotte M, Landri S, Lepage A, Fulop T, Ramassamy C (2019) Circulating and extracellular vesicles levels of N-(1-carboxymethyl)-L-lysine (CML) differentiate early to moderate Alzheimer's disease. J Alzheimers Dis 69:751-762.

Hajishengallis G, Reis ES, Mastellos DC, Ricklin D, Lambris JD (2017) Novel mechanisms and functions of complement. Nat Immunol 18:1288-1298.

Hornung S, Dutta S, Bitan G (2020) CNS-derived blood exosomes as a promising source of biomarkers: opportunities and challenges. Front Mol Neurosci 13:38.

Huber CM, Yee C, May T, Dhanala A, Mitchell CS (2018) Cognitive decline in preclinical Alzheimer's disease: amyloid-beta versus tauopathy. J Alzheimers Dis 61:265-281.

Ihara Y, Morishima-Kawashima M, Nixon R (2012) The ubiquitin-proteasome system and the autophagic-lysosomal system in Alzheimer disease. Cold Spring Harb Perspect Med 2:a006361.

Iqbal K, Liu F, Gong CX (2016) Tau and neurodegenerative disease: the story so far. Nat Rev Neurol 12:15-27.

Jack CJ, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R (2018) NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement 14:535-562.

Janelidze S, Hertze J, Zetterberg H, Landqvist WM, Santillo A, Blennow K, Hansson O (2016) Cerebrospinal fluid neurogranin and YKL-40 as biomarkers of Alzheimer's disease. Ann Clin Transl Neurol 3:12-20.

Janelidze S, Stomrud E, Smith R, Palmqvist S, Mattsson N, Airey DC, Proctor NK, Chai X, Shcherbinin S, Sims JR, Triana-Baltzer G, Theunis C, Slemmon R, Mercken M, Kolb H, Dage JL, Hansson O (2020) Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. Nat Commun 11:1683.

Jeon SG, Kang M, Kim YS, Kim DH, Nam DW, Song EJ, Mook-Jung I, Moon M (2018) Intrahippocampal injection of a lentiviral vector expressing neurogranin enhances cognitive function in 5XFAD mice. Exp Mol Med 50:e461.

Jia L, Qiu Q, Zhang H, Chu L, Y D, Zhang J, Zhou C, Liang F, Shi S, Wang S, Qin W, Wang Q, Li F, Wang Q, Li Y, Shen L, Wei Y, Jia J (2019) Concordance between the assessment of Abeta42, T-tau, and P-T181-tau in peripheral blood neuronal-derived exosomes and cerebrospinal fluid. Alzheimers Dement 15:1071-1080.

Jia L, Zhu M, Kong C, Pang Y, Zhang H, Qiu Q, Wei C, Tang Y, Wang Q, Li Y, Li T, Li F, Wang Q, Li Y, Wei Y, Jia J (2020a) Blood neuro-exosomal synaptic proteins predict Alzheimer's disease at the asymptomatic stage. Alzheimers Dement doi: 10.1002/alz.12166.

Jia L, Du Y, Chu L, Zhang Z, Li F, Lyu D, Li Y, Li Y, Zhu M, Jiao H, Song Y, Shi Y, Zhang H, Gong M, Wei C, Tang Y, Fang B, Guo D, Wang F, Zhou A, et al. (2020b) Prevalence, risk factors, and management of dementia and mild cognitive impairment in adults aged 60 years or older in China: a cross-sectional study. Lancet Public Health 5:e661-e671.

Johnson ECB, Dammer EB, Duong DM, Ping L, Zhou M, Yin L, Higginbotham LA, Guajardo A, White B, Troncoso JC, Thambisetty M, Montine TJ, Lee EB, Trojanowski JQ, Beach TG, Reiman EM, Haroutunian V, Wang M, Schadt E, Zhang B, et al. (2020) Large-scale proteomic analysis of Alzheimer's disease brain and cerebrospinal fluid reveals early changes in energy metabolism associated with microglia and astrocyte activation. Nat Med 26:769-780.

Kalluri R, LeBleu VS (2020) The biology, function, and biomedical applications of exosomes. Science 367:eaau6977.

Kapogiannis D, Boxer A, Schwartz JB, Abner EL, Biragyn A, Masharani U, Frassetto L, Petersen RC, Miller BL, Goetzl EJ (2015) Dysfunctionally phosphorylated type 1 insulin receptor substrate in neural-derived blood exosomes of preclinical Alzheimer's disease. FASEB J 29:589-596.

Kapogiannis D, Mustapic M, Shardell MD, Berkowitz ST, Diehl TC, Spangler RD, Tran J, Lazaropoulos MP, Chawla S, Gulyani S, Eitan E, An Y, Huang CW, Oh ES, Lyketsos CG, Resnick SM, Goetzl EJ, Ferrucci L (2019) Association of extracellular vesicle biomarkers with Alzheimer disease in the baltimore longitudinal study of aging. JAMA Neurol 76:1340-1351.

Kerr JS, Adriaanse BA, Greig NH, Mattson MP, Cader MZ, Bohr VA, Fang EF (2017) Mitophagy and Alzheimer's disease: cellular and molecular mechanisms. Trends Neurosci 40:151-166.

Kim KY, Shin KY, Chang KA (2021) Brain-derived exosomal proteins as effective biomarkers for Alzheimer's disease: a systematic review and meta-analysis. Biomolecules 11:980.

Kwak SS, Washicosky KJ, Brand E, von Maydell D, Aronson J, Kim S, Capen DE, Cetinbas M, Sadreyev R, Ning S, Bylykbashi E, Xia W, Wagner SL, Choi SH, Tanzi RE, Kim DY (2020) Amyloid-beta42/40 ratio drives tau pathology in 3D human neural cell culture models of Alzheimer's disease. Nat Commun 11:1377.

Lee JD, Coulthard LG, Woodruff TM (2019) Complement dysregulation in the central nervous system during development and disease. Semin Immunol 45:101340.

Lewczuk P, Riederer P, O'Bryant SE, Verbeek MM, Dubois B, Visser PJ, Jellinger KA, Engelborghs S, Ramirez A, Parnetti L, Jack CR Jr, Teunissen CE, Hampel H, Lleó A, Jessen F, Glodzik L, de Leon MJ, Fagan AM, Molinuevo JL, Jansen WJ, et al. (2018) Cerebrospinal fluid and blood biomarkers for neurodegenerative dementias: an update of the consensus of the task force on biological markers in psychiatry of the world federation of societies of biological psychiatry. World J Biol Psychiatry 19:244-328.

Li F, Xie XY, Sui XF, Wang P, Chen Z, Zhang JB (2020a) Profile of pathogenic proteins and micrornas in plasma-derived extracellular vesicles in Alzheimer's disease: a pilot study. Neuroscience 432:240-246.

Li L, Lai M, Cole S, Le Novere N, Edelstein SJ (2020b) Neurogranin stimulates Ca^{2+}/calmodulin-dependent kinase II by suppressing calcineurin activity at specific calcium spike frequencies. PLoS Comput Biol 16:e1006991.

Lista S, Hampel H (2017) Synaptic degeneration and neurogranin in the pathophysiology of Alzheimer's disease. Expert Rev Neurother 17:47-57.

Liu W, Lin H, He X, Chen L, Dai Y, Jia W, Xue X, Tao J, Chen L (2020) Neurogranin as a cognitive biomarker in cerebrospinal fluid and blood exosomes for Alzheimer's disease and mild cognitive impairment. Transl Psychiatry 10:125.

Lu T, Aron L, Zullo J, Pan Y, Kim H, Chen Y, Yang TH, Kim HM, Drake D, Liu XS, Bennett DA, Colaiacovo MP, Yankner BA (2014) REST and stress resistance in ageing and Alzheimer's disease. Nature 507:448-454.

Lugli G, Cohen AM, Bennett DA, Shah RC, Fields CJ, Hernandez AG, Smalheiser NR (2015) Plasma exosomal miRNAs in persons with and without alzheimer disease: altered expression and prospects for biomarkers. PLoS One 10:e0139233.

Mattsson-Carlgren N, Andersson E, Janelidze S, Ossenkoppele R, Insel P, Strandberg O, Zetterberg H, Rosen HJ, Rabinovici G, Chai X, Blennow K, Dage JL, Stomrud E, Smith R, Palmqvist S, Hansson O (2020) Aβ deposition is associated with increases in soluble and phosphorylated tau that precede a positive Tau PET in Alzheimer's disease. Sci Adv 6:eaaz2387.

McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CJ, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7:263-269.

Milà-Alomà M, Salvadó G, Gispert JD, Vilor-Tejedor N, Grau-Rivera O, Sala-Vila A, Sánchez-Benavides G, Arenaza-Urquijo EM, Crous-Bou M, González-de-Echávarri JM, Minguillon C, Fauria K, Simon M, Kollmorgen G, Zetterberg H, Blennow K, Suárez-Calvet M, Molinuevo JL (2020) Amyloid beta, tau, synaptic, neurodegeneration, and glial biomarkers in the preclinical stage of the Alzheimer's continuum. Alzheimers Dement 16:1358-1371.

Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 339:b2535.

Moloney AM, Griffin RJ, Timmons S, O'Connor R, Ravid R, O'Neill C (2010) Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. Neurobiol Aging 31:224-243.

Morgan BP, Harris CL (2015) Complement, a target for therapy in inflammatory and degenerative diseases. Nat Rev Drug Discov 14:857-877.

Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Dore V, Fowler C, Li QX, Martins R, Rowe C, Tomita T, Matsuzaki K, Ishii K, Ishii K, Arahata Y, Iwamoto S, Ito K, Tanaka K, Masters CL, Yanagisawa K (2018) High performance plasma amyloid-beta biomarkers for Alzheimer's disease. Nature 554:249-254.

Nam E, Lee YB, Moon C, Chang KA (2020) Serum tau proteins as potential biomarkers for the assessment of Alzheimer's disease progression. Int J Mol Sci 21:5007.

Neal BS, Lack SD, Lankhorst NE, Raye A, Morrissey D, van Middelkoop M (2019) Risk factors for patellofemoral pain: a systematic review and meta-analysis. Br J Sports Med 53:270-281.

Nie C, Sun Y, Zhen H, Guo M, Ye J, Liu Z, Yang Y, Zhang X (2020) differential expression of plasma exo-miRNA in neurodegenerative diseases by next-generation sequencing. Front Neurosci 14:438.

Olsson B, Lautner R, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, Hölttä M, Rosén C, Olsson C, Strobel G, Wu E, Dakin K, Petzold M, Blennow K, Zetterberg H (2016) CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol 15:673-684.

Perrotte M, Haddad M, Le Page A, Frost EH, Fulöp T, Ramassamy C (2020) Profile of pathogenic proteins in total circulating extracellular vesicles in mild cognitive impairment and during the progression of Alzheimer's disease. Neurobiol Aging 86:102-111.

Reza-Zaldivar EE, Hernández-Sapiéns MA, Minjarez B, Gutiérrez-Mercado YK, Márquez-Aguirre AL, Canales-Aguirre AA (2018) Potential effects of MSC-derived exosomes in neuroplasticity in Alzheimer's disease. Front Cell Neurosci 12:317.

Robinson M, Lee BY, Hane FT (2017) Recent progress in Alzheimer's disease research, part 2: genetics and epidemiology. J Alzheimers Dis 57:317-330.

Rodriguez-Rodriguez P, Sandebring-Matton A, Merino-Serrais P, Parrado-Fernandez C, Rabano A, Winblad B, Avila J, Ferrer I, Cedazo-Minguez A (2017) Tau hyperphosphorylation induces oligomeric insulin accumulation and insulin resistance in neurons. Brain 140:3269-3285.

Sasaguri H, Nilsson P, Hashimoto S, Nagata K, Saito T, De Strooper B, Hardy J, Vassar R, Winblad B, Saido TC (2017) APP mouse models for Alzheimer's disease preclinical studies. EMBO J 36:2473-2487.

Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S, Van der Flier WM (2016) Alzheimer's disease. Lancet 388:505-517.

Schwagerl AL, Mohan PS, Cataldo AM, Vonsattel JP, Kowall NW, Nixon RA (1995) Elevated levels of the endosomal-lysosomal proteinase cathepsin D in cerebrospinal fluid in Alzheimer disease. J Neurochem 64:443-446.

Serpente M, Fenoglio C, D'Anca M, Arcaro M, Sorrentino F, Visconte C, Arighi A, Fumagalli GG, Porretti L, Cattaneo A, Ciani M, Zanardini R, Benussi L, Ghidoni R, Scarpini E, Galimberti D (2020) miRNA profiling in plasma neural-derived small extracellular vesicles from patients with Alzheimer's disease. Cells 9:1443.

Solje E, Benussi A, Buratti E, Remes AM, Haapasalo A, Borroni B (2021) State-of-the-art methods and emerging fluid biomarkers in the diagnostics of dementia-a short review and diagnostic algorithm. Diagnostics 11:788.

Stang A (2010) Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 25:603-605.

Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB (2000) Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 283:2008-2012.

Sun R, Wang H, Shi Y, Sun Z, Jiang H, Zhang J (2020) Changes in the morphology, number, and pathological protein levels of plasma exosomes may help diagnose Alzheimer's disease. J Alzheimers Dis 73:909-917.

Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, Fuino RL, Kawaguchi KR, Samoyedny AJ, Wilson RS, Arvanitakis Z, Schneider JA, Wolf BA, Bennett DA, Trojanowski JQ, Arnold SE (2012) Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. J Clin Invest 122:1316-1338.

Tenner AJ (2020) Complement-mediated events in Alzheimer's disease: mechanisms and potential therapeutic targets. J Immunol 204:306-315.

Tiwari S, Atluri V, Kaushik A, Yndart A, Nair M (2019) Alzheimer's disease: pathogenesis, diagnostics, and therapeutics. Int J Nanomedicine 14:5541-5554.

van der Kant R, Goldstein L, Ossenkoppele R (2020) Amyloid-β-independent regulators of tau pathology in Alzheimer disease. Nat Rev Neurosci 21:21-35.

Wang D, Wang P, Bian X, Xu S, Zhou Q, Zhang Y, Ding M, Han M, Huang L, Bi J, Jia Y, Xie Z (2020) Elevated plasma levels of exosomal BACE1-AS combined with the volume and thickness of the right entorhinal cortex may serve as a biomarker for the detection of Alzheimer's disease. Mol Med Rep 22:227-238.

Wang H, Sui H, Zheng Y, Jiang Y, Shi Y, Liang J, Zhao L (2019) Curcumin-primed exosomes potently ameliorate cognitive function in AD mice by inhibiting hyperphosphorylation of the Tau protein through the AKT/GSK-3β pathway. Nanoscale 11:7481-7496.

Wang L, Zhang L (2020) Circulating Exosomal miRNA as diagnostic biomarkers of neurodegenerative diseases. Front Mol Neurosci 13:53.

Wellington H, Paterson RW, Portelius E, Tornqvist U, Magdalinou N, Fox NC, Blennow K, Schott JM, Zetterberg H (2016) Increased CSF neurogranin concentration is specific to Alzheimer disease. Neurology 86:829-835.

Wellington H, Paterson RW, Suarez-Gonzalez A, Poole T, Frost C, Sjobom U, Slattery CF, Magdalinou NK, Lehmann M, Portelius E, Fox NC, Blennow K, Zetterberg H, Schott JM (2018) CSF neurogranin or tau distinguish typical and atypical Alzheimer disease. Ann Clin Transl Neurol 5:162-171.

Wildsmith KR, Schauer SP, Smith AM, Arnott D, Zhu Y, Haznedar J, Kaur S, Mathews WR, Honigberg LA (2014) Identification of longitudinally dynamic biomarkers in Alzheimer's disease cerebrospinal fluid by targeted proteomics. Mol Neurodegener 9:22.

Winston CN, Goetzl EJ, Akers JC, Carter BS, Rockenstein EM, Galasko D, Masliah E, Rissman RA (2016) Prediction of conversion from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. Alzheimers Dement 3:63-72.

Winston CN, Goetzl EJ, Baker LD, Vitiello MV, Rissman RA (2018) Growth hormone-releasing hormone modulation of neuronal exosome biomarkers in mild cognitive impairment. Alzheimers Dis 66:971-981.

Winston CN, Goetzl EJ, Schwartz JB, Elahi FM, Rissman RA (2019) Complement protein levels in plasma astrocyte-derived exosomes are abnormal in conversion from mild cognitive impairment to Alzheimer's disease dementia. Alzheimers Dement 11:61-66.

Wood MJ, O'Loughlin AJ, Samira L (2011) Exosomes and the blood-brain barrier: implications for neurological diseases. Ther Deliv 2:1095-1099.

Xian P, Hei Y, Wang R, Wang T, Yang J, Li J, Z D, Liu Z, Baskys A, Liu W, Wu S, Long Q (2019) Mesenchymal stem cell-derived exosomes as a nanotherapeutic agent for amelioration of inflammation-induced astrocyte alterations in mice. Theranostics 9:5956-5975.

Xin SH, Tan L, Cao X, Yu JT, Tan L (2018) Clearance of amyloid beta and Tau in Alzheimer's disease: from mechanisms to therapy. Neurotox Res 34:733-748.

Xing W, Gao W, Lv X, Xu X, Zhang Z, Yan J, Mao G, Bu Z (2021) The diagnostic value of exosome-derived biomarkers in Alzheimer's disease and mild cognitive impairment: a meta-analysis. Front Aging Neurosci 13:637218.

Yang TT, Liu CG, Gao SC, Zhang Y, Wang PC (2018) The Serum exosome derived microRNA-135a, -193b, and-384 were potential Alzheimer's disease biomarkers. Biomed Environ Sci 31:87-96.

Yin Q, Ji X, Lv R, Pei JJ, Y D, Shen C, Hou X (2020) Targetting exosomes as a new biomarker and therapeutic approach for Alzheimer's disease. Clin Interv Aging 15:195-205.

Zhang N, Gu D, Meng M, Gordon ML (2020) TDP-43 Is elevated in plasma neuronal-derived exosomes of patients with Alzheimer's disease. Front Aging Neurosci 12:166.

Zhao A, Li Y, Yan Y, Qiu Y, Li B, Xu W, Wang Y, Liu J, Deng Y (2020) Increased prediction value of biomarker combinations for the conversion of mild cognitive impairment to Alzheimer's dementia. Transl Neurodegener 9:30.

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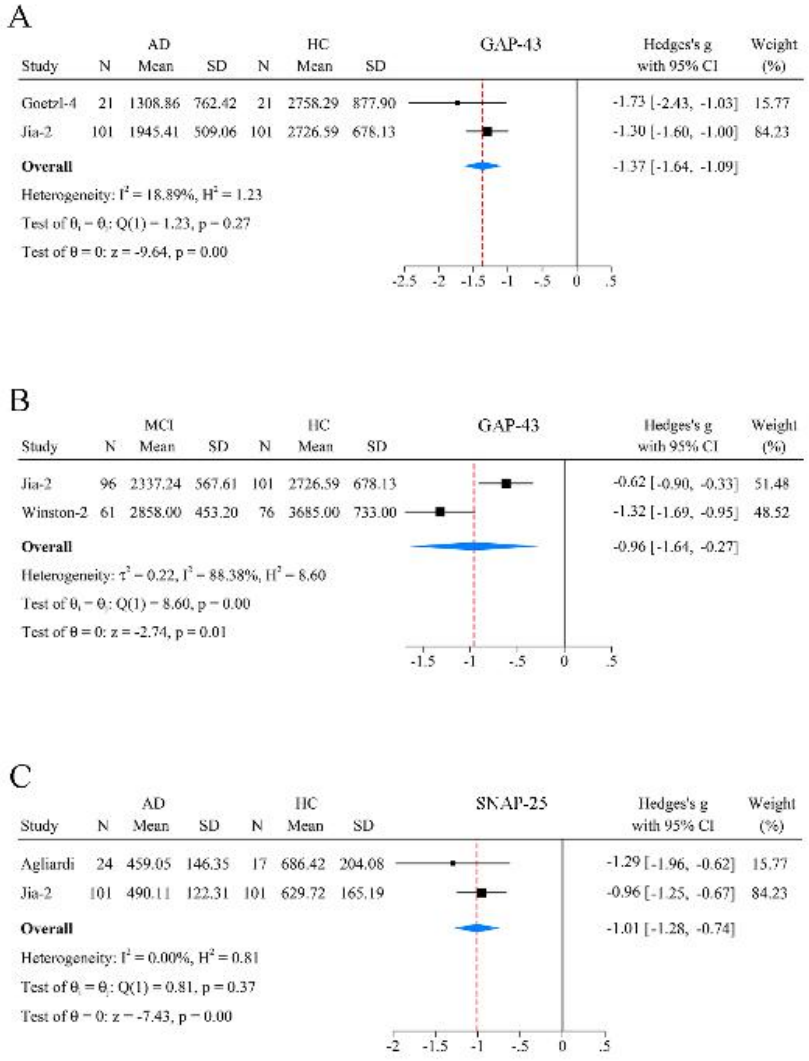
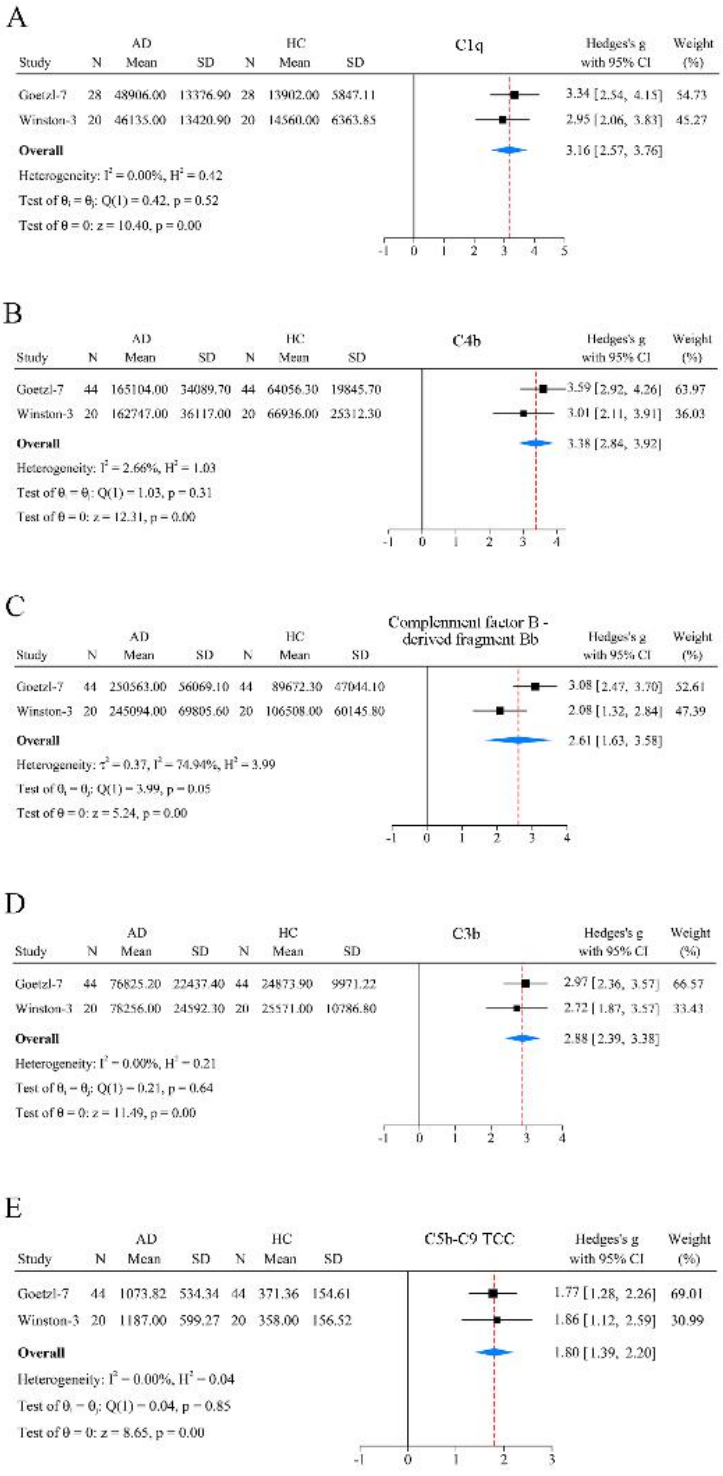
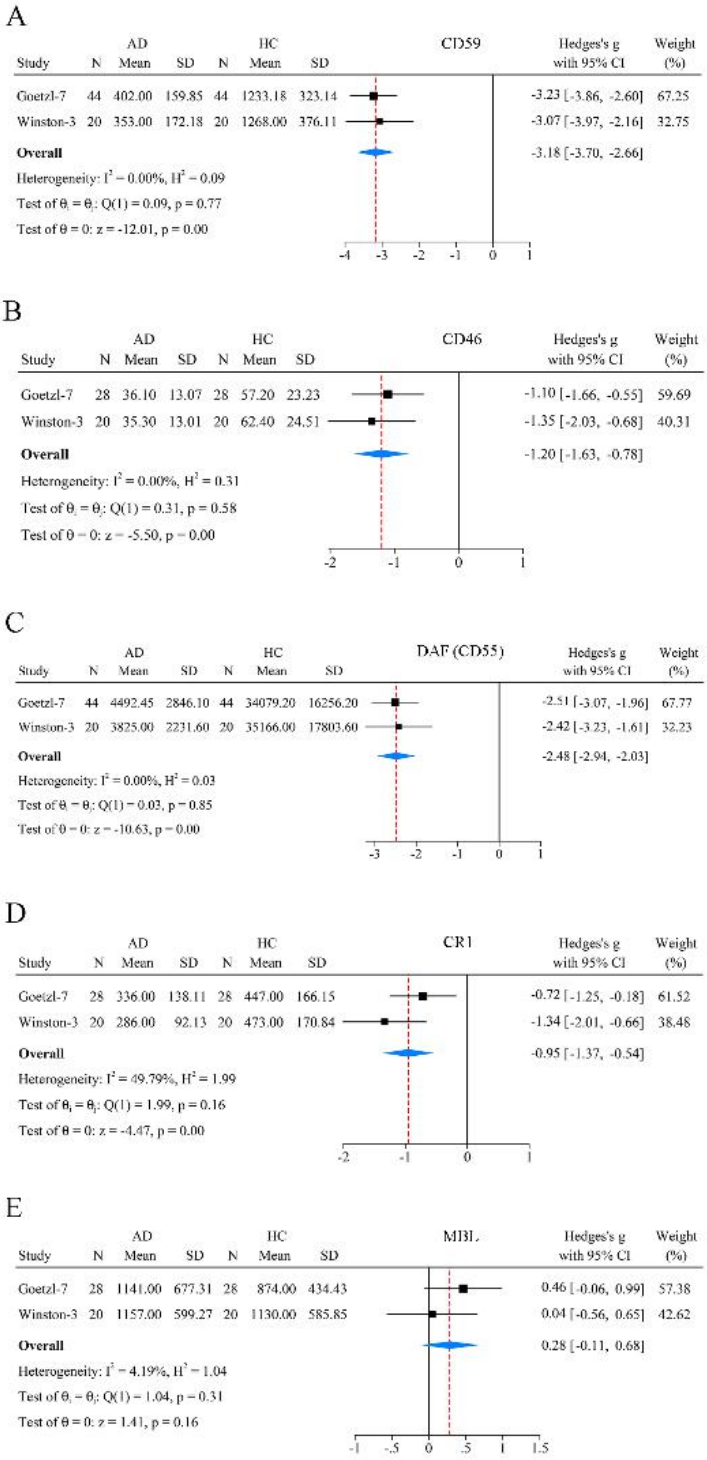


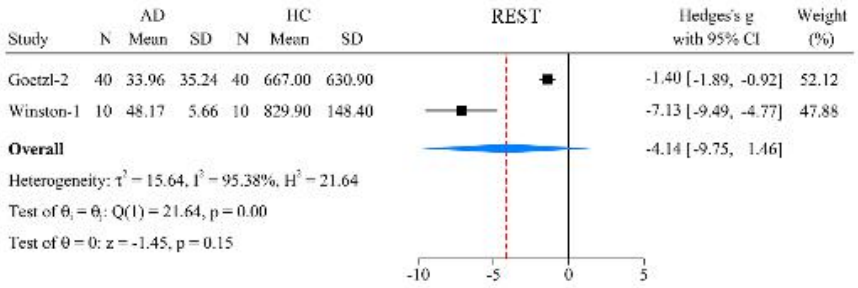
Additional Figure 1 Results of meta-analysis in GAP-43 and SNAP-25 levels among patients with AD, MCI and HCs.
(A) The SMD and corresponding 95% CIs of the GAP-43 level between patients with AD and HC subjects were shown in the forest plot. (B) The SMD and corresponding 95% CIs of the GAP-43 level between patients with MCI and HC subjects were shown in the forest plot. **(**C) The SMD and corresponding 95% CIs of the SNAP-25 level between patients with AD and HC subjects were shown in the forest plot. The sign after the author means that there are several authors with the same last name or some studies with the same first author, and the number represents the order of the article, which aims to distinguish these studies. AD: Alzheimer's disease; CI: confidence interval; GAP-43: grown-associated protein 43; HC: healthy control; MCI: mild cognitive impairment; SMD: standard mean difference; SNAP-25: synaptosomal associated protein-25.



Additional Figure 2 Results of meta-analysis in levels of complement effector proteins between patients with AD and HCs.
(A) The SMD and corresponding 95% CIs of the C1q level between patients with AD and HC subjects were shown in the forest plot. (B) The SMD and corresponding 95% CIs of the C4b level between patients with AD and HC subjects were shown in the forest plot. (C) The SMD and corresponding 95% CIs of the complement factor B-derived fragment Bb level between patients with AD and HC subjects were shown in the forest plot. (D) The SMD and corresponding 95% CIs of the C3b level between patients with AD and HC subjects were shown in the forest plot. (E) The SMD and corresponding 95% CIs of the C5b-C9 TCC level between patients with AD and HC subjects were shown in the forest plot. The sign after the author means that there are several authors with the same last name or some studies with the same first author, and the number represents the order of the article, which aims to distinguish these studies. AD: Alzheimer's disease; CI: confidence interval; C1q: complement 1q; C5b-C9 TCC: C5b-C9 terminal complement complex; HC: healthy control; SMD: standard mean difference.

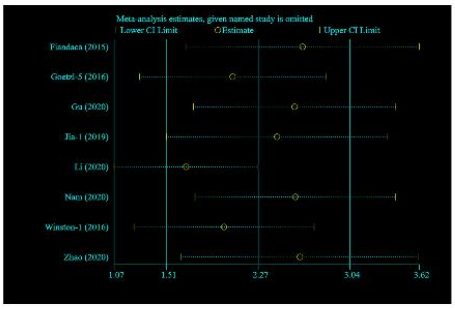


Additional Figure 3 Results of meta-analysis in levels of complement regulatory proteins between patients with AD and HCs. (A) The SMD and corresponding 95% CIs of the CD59 level between patients with AD and HC subjects were shown in the forest plot. (B) The SMD and corresponding 95% CIs of the CD46 level between patients with AD and HC subjects were shown in the forest plot. (C) The SMD and corresponding 95% CIs of the DAF(CD55) level between patients with AD and HC subjects were shown in the forest plot. (D) The SMD and corresponding 95% CIs of the CR1 level between patients with AD and HC subjects were shown in the forest plot. (E) The SMD and corresponding 95% CIs of the MBL level between patients with AD and HC subjects were shown in the forest plot. The sign after the author means that there are several authors with the same last name or some studies with the same first author, and the number represents the order of the article, which aims to distinguish these studies. AD: Alzheimer's disease; CI: confidence interval; CR1: complement receptor 1; DAF: decay-accelerating factor; HC: healthy control; MBL: mannose-binding lectin; SMD: standard mean difference.

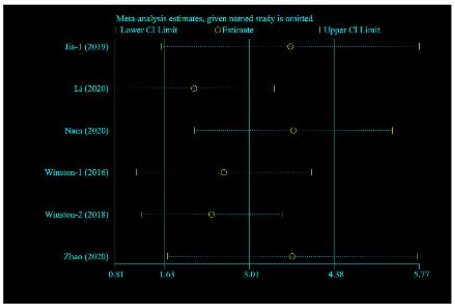


Additional Figure 4 Results of meta-analysis in the REST level between patients with AD and HCs.
The SMD and corresponding 95% CIs of the REST level between patients with AD and HC subjects were shown in the forest plot. The sign after the author means that there are several authors with the same last name or some studies with the same first author, and the number represents the order of the article, which aims to distinguish these studies. AD: Alzheimer's disease; CI: confidence interval; HC: healthy control; REST: repressor element 1-silencing transcription; SMD: standard mean difference.

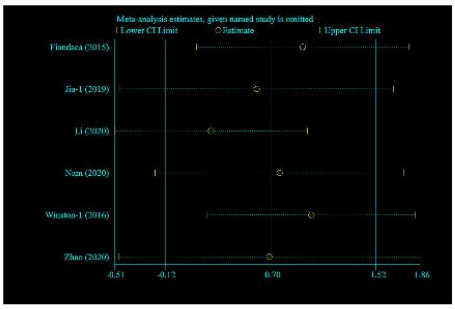
A $A\beta_{1-42}$ AD vs. HC



B $A\beta_{1-42}$ MCI vs. HC

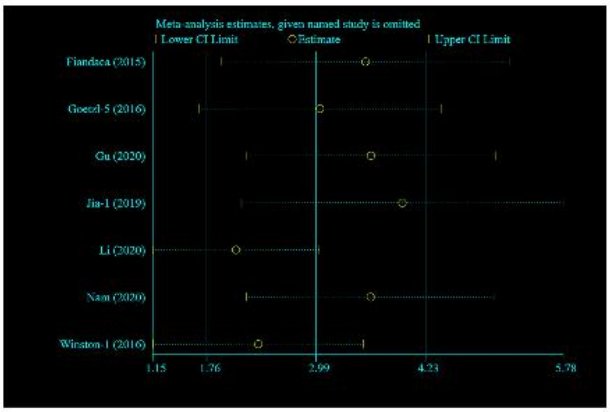


C $A\beta_{1-42}$ AD vs. MCI

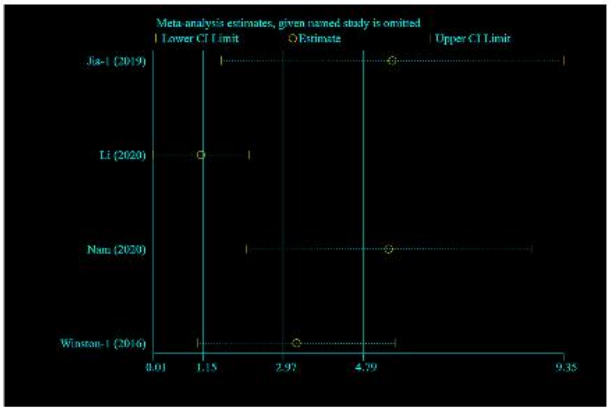


Additional Figure 5 Results of sensitivity analysis in $A\beta_{1-42}$ levels among patients with AD, MCI and HCs.
(A) The effect of a single study on meta-analysis estimates in the $A\beta_{1-42}$ level between patients with AD and HC subjects. (B) The effect of a single study on meta-analysis estimates in the $A\beta_{1-42}$ level between patients with MCI and HC subjects. **(**C) The effect of a single study on meta-analysis estimates in the $A\beta_{1-42}$ level between patients with AD and MCI. The sign after the author means that there are several authors with the same last name or some studies with the same first author, and the number represents the order of the article, which aims to distinguish these studies. AD: Alzheimer's disease; HC: healthy control; MCI: mild cognitive impairment.

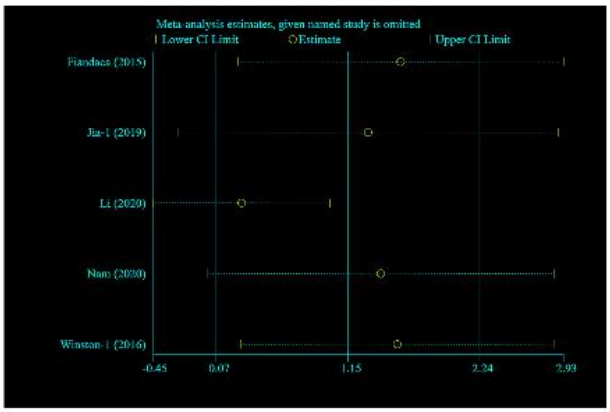
A P-T181-tau AD vs. HC



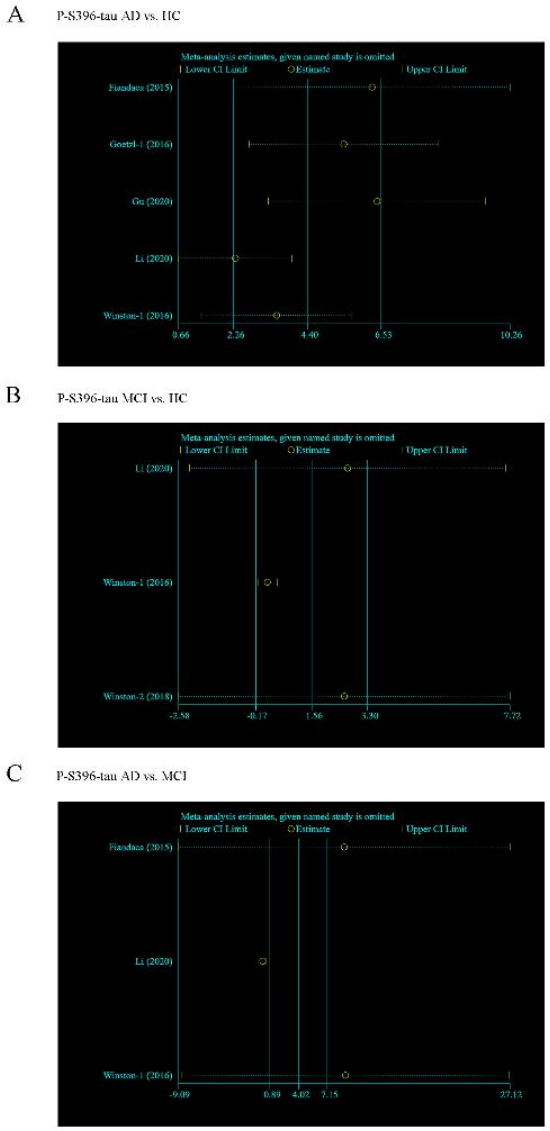
B P-T181-tau MCI vs. HC



C P-T181-tau AD vs. MCI

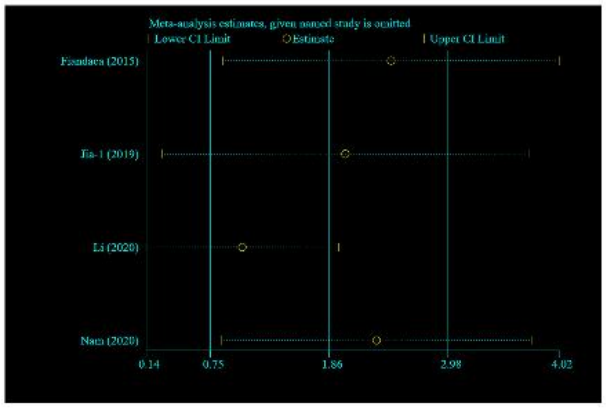


Additional Figure 6 Results of sensitivity analysis in P-T181-tau levels among patients with AD, MCI and HCs.
(A) The effect of a single study on meta-analysis estimates in the P-T181-tau level between patients with AD and HC subjects. (B) The effect of a single study on meta-analysis estimates in the P-T181-tau level between patients with MCI and HC subjects. **(**C) The effect of a single study on meta-analysis estimates in the P-T181-tau level between patients with AD and MCI. The sign after the author means that there are several authors with the same last name or some studies with the same first author, and the number represents the order of the article, which aims to distinguish these studies. AD: Alzheimer's disease; HC: healthy control; MCI: mild cognitive impairment; P-T181-tau: phosphorylated tau (threonine 181).

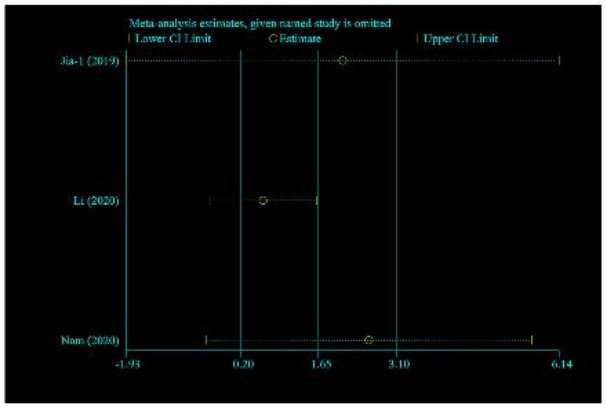


Additional Figure 7 Results of sensitivity analysis in P-S396-tau levels among patients with AD, MCI and HCs.
(A) The effect of a single study on meta-analysis estimates in the P-S396-tau level between patients with AD and HC subjects. (B) The effect of a single study on meta-analysis estimates in the P-S396-tau level between patients with MCI and HC subjects. **(**C) The effect of a single study on meta-analysis estimates in the P-S396-tau level between patients with AD and MCI. The sign after the author means that there are several authors with the same last name or some studies with the same first author, and the number represents the order of the article, which aims to distinguish these studies. AD: Alzheimer's disease; HC: healthy control; MCI: mild cognitive impairment; P-S396-tau: phosphorylated tau (serine 396).

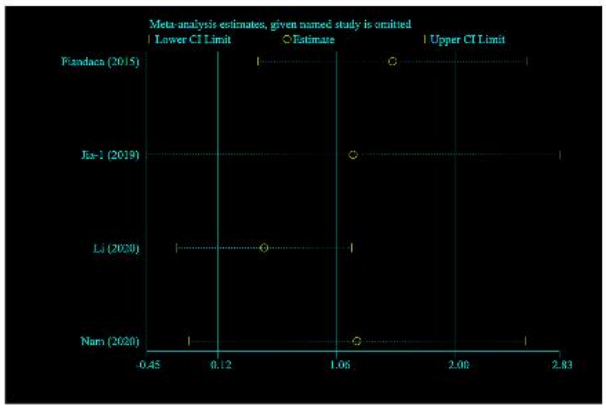
A T-tau AD vs. HC



B T-tau MCI vs. HC

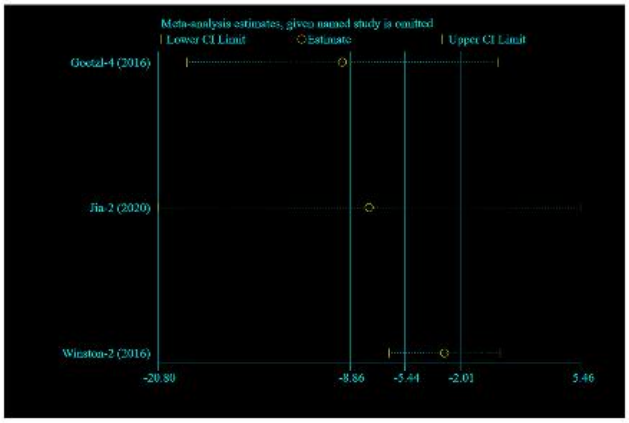


C T-tau AD vs. MCI

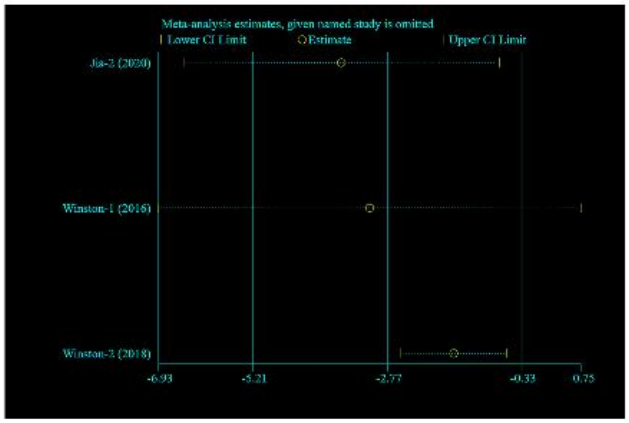


Additional Figure 8 Results of sensitivity analysis in T-tau levels among patients with AD, MCI and HCs.
(A) The effect of a single study on meta-analysis estimates in the T-tau level between patients with AD and HC subjects. (B) The effect of a single study on meta-analysis estimates in the T-tau level between patients with MCI and HC subjects. **(**C) The effect of a single study on meta-analysis estimates in the T-tau level between patients with AD and MCI. The sign after the author means that there are several authors with the same last name or some studies with the same first author, and the number represents the order of the article, which aims to distinguish these studies. AD: Alzheimer's disease; HC: healthy control; MCI: mild cognitive impairment; T-tau: total tau.

A Neurogranin AD vs. HC



B Neurogranin MCI vs. HC



Additional Figure 9 Results of sensitivity analysis in neurogranin levels among patients with AD, MCI and HCs.
(A) The effect of a single study on meta-analysis estimates in the T-tau level between patients with AD and HC subjects. (B) The effect of a single study on meta-analysis estimates in the T-tau level between patients with MCI and HC subjects. The sign after the author means that there are several authors with the same last name or some studies with the same first author, and the number represents the order of the article, which aims to distinguish these studies. AD: Alzheimer's disease; HC: healthy control; MCI: mild cognitive impairment.