Research Square

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Blood neuroexosomal mitochondrial proteins predict Alzheimer's disease in diabetes mellitus

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Abstract

Background

There is accumulating evidence that mitochondrial dysfunction is associated with the contribution of diabetes to Alzheimer's disease (AD) progression. Neuronal mitochondrial proteins found in plasma neuronal-derived exosomes(NDEs) at levels that reflect those in brain neurons. Here, we tested the performance of mitochondrial proteins in plasma NDEs to predict cognitive decline and brain injury in diabetic participants.

Methods

The type 2 diabetes mellitus (T2DM) participants in the study included 41 cognitively normal controls, 97 individuals with mild cognitive impairment (MCI) (68 individuals with stable MCI; 29 individuals with progressive MCI) and 36 patients with AD dementia. Plasma neuroexosomal proteins were measured by ELISA kits. Spearman's correlation was used to test associations between mitochondrial proteins and other core biomarkers of AD in plasma neuronal-derived exosomes. Diagnostic accuracy (the area under the curve (AUC)) for progressive MCI and AD was obtained for mitochondrial proteins using receiver operating curve (ROC) analyses. The associations of mitochondrial proteins with the conversion from MCI to AD were assessed by Cox proportional hazard regression analysis.

Results

Plasma neuroexosomal NADH ubiquinone oxidoreductase core subunit S3 (NDUFS3) and succinate dehydrogenase complex subunit B (SDHB) levels were significantly lower in T2DM patients with AD dementia (232.7 ± 63.4, 1360.7 ± 328.5, pg/ml) and progressive MCI (274.4 ± 78.6, 1536.7 ± 342.8, pg/ml) than in cognitively normal subjects (333.9 ± 96.7, 2050.4 ± 628.9, pg/ml) ($P < 0.001$ for both groups). We also found that plasma neuroexosomal NDUFS3 and SDHB levels were lower in progressive MCI (274.4 ± 78.6 pg/ml, 1536.7 ± 342.8 pg/ml) than in stable MCI (319.9 ± 109.8 pg/ml, $P < 0.05$; 1824.7 ± 606.4 pg/ml, $P < 0.05$) subjects. Both plasma neuroexosomal NDUFS3 and SDHB offer diagnostic utility for AD. Low plasma neuroexosomal SDHB levels significantly predicted conversion from MCI to AD. In addition, low mitochondrial proteins levels were associated with the rate of hippocampal and gray matter atrophy, reduced AD signature cortical thickness in progressive MCI over the follow-up period.

Conclusions

These data suggest that both plasma neuroexosomal NDUFS3 and SDHB are already increased at the early clinical stage of AD, and indicate the promise of plasma neuroexosomal mitochondrial proteins as

diagnostic and prognostic biomarkers for the earliest symptomatic stage of AD in diabetic participants .

Background

The incidence of type 2 diabetes mellitus (T2DM) is increasing worldwide, and the disease, common in older adults, has become a significant public health problem. Cognitive dysfunction, including mild cognitive impairment (MCI) and dementia, is increasingly recognized as an important comorbidity and complication of diabetes that affects an individual's well-being and diabetes management. Numerous epidemiological studies have demonstrated that T2DM is associated with accelerated age-related cognitive decline, a higher incidence of MCI and a nearly 2-fold increased incident risk of Alzheimer's disease (AD), even after adjusting for vascular risk factors [1, 2]. Furthermore, diabetes and prediabetes substantially accelerate the progression from MCI to AD [3].

Exosomes are vesicle mediators of intercellular communication and of the maintenance of cellular homeostasis. Cells from the central nervous system use exosomes as a strategy not only to eliminate membranes, toxic proteins, and RNA species but also to mediate short and long cell-to-cell communication as carriers of important messengers and signals [4]. Exosomes cross the blood brain barrier and are detectable in the peripheral circulation. The levels of plasma exosomal biomarkers reflect pathological brain changes. Jia et al. reported that the levels of amyloid-β (Aβ), total tau (T-tau), and tau phosphorylated at threonine 181 (P-T181-tau) in blood neuronal-derived exosomes were highly correlated with their levels in the cerebrospinal fluid in AD and amnestic mild cognitive impairment (aMCI) patients [5]. Diabetes elicits AD-like brain changes linked with cognitive decline and neurodegeneration, such as elevated tau expression and phosphorylation, accumulation of Aβ and mitochondrial and synaptic dysfunction [6–8]. As a common denominator in AD and diabetes, mitochondrial dysfunction has also emerged as a possible mechanistic bridge between these two pathologies [9, 10]. Increased evidence has indicated that mitochondrial and synaptic dysfunction is an early pathological feature of AD [11]. Some mitochondrial proteins (subunits of electron transport chain complex I and complex II, such as NDUFS3 and SDHB) were identified in circulating exosomes from older adults with Parkinson’s disease [12]. However, it remains unknown whether plasma neuroexosomal mitochondrial changes occur in the early clinical stage of AD among T2DM patients and whether plasma neuroexosomal mitochondrial proteins are correlated with other core features of AD, such as cognitive decline, amyloid-β pathology, and structural brain changes. In this study, we investigated the capacity of plasma neuroexosomal mitochondrial proteins to detect preclinical AD before cognitive impairment in T2DM patients and analyzed the correlation between exosomal biomarkers and changes in cognition, in AD pathogenic proteins, such as Aβ42, and in tau proteins in plasma neural-derived exosomes, and on MRI over time.

Materials And Methods

Study subjects

We enrolled type 2 diabetic patients with mild cognitive impairment (MCI) at the Endocrinology Clinic of Weihai Municipal Hospital between January 2017 and June 2017. All subjects provided written informed consent before inclusion, and the study followed the Declaration of Helsinki and was approved by the ethics committee of Weihai Municipal Hospital. T2DM was diagnosed according to the 1999 World Health Organization criteria. The criteria for MCI included the presence of a subjective memory complaint, with a Mini-Mental State Examination (MMSE) score between 24 and 30, a clinical dementia rating (CDR) of 0.5, preserved activities of daily living, and an absence of dementia [13]. All subjects were included in the baseline population based on the following criteria: (1) T2DM patients with a disease duration of more than 3 years; (2) patients who were 55 years old and older; and (3) long-term residents of Weihai City. The exclusion criteria included cortical stroke, seizure, brain surgery, a history of traumatic brain injury, a concomitant neurologic disorder potentially affecting cognitive function (e.g., Parkinson disease), and being unable to comply with the study assessment. Subjects with MCI and depressive disorder were also excluded [14].

Data on demographic characteristics and vascular risk factors were collected. The patients underwent neuropsychological testing (including the MMSE, Montreal Cognitive Assessment (MOCA), Clinical Dementia Rating (CDR), the 17-item version of the Hamilton Depression Rating Scale (HAM-D), and the ADL scale) and brain magnetic resonance imaging (MRI) at baseline and during the follow-up period.

All T2DM patients with MCI were followed up for more than 24 months (mean 40.6 months). The point of dementia conversion was determined by two neurologists based on criteria modified from the DSM-IV [15]. The subjects with dementia were further subjected to brain MRI. Dementia of the Alzheimer's type was diagnosed based on the NINDS-ADRDA criteria [16]. The diagnosis of vascular dementia (VaD) was based on the criteria of the NINDS-AIREN [17]. AD patients were included in the present study. At the time of the last available assessment, MCI patients were classified as having progressive mild cognitive impairment (pMCI) if they progressed to developing AD or as having stable mild cognitive impairment (sMCI) if their diagnosis remained MCI. We excluded subjects who were diagnosed with MCI at baseline but reverted to cognitively normal (CN) during follow-up.

We also recruited age-, sex-, and education-matched type 2 diabetes patients with cognitively normal and AD patients. The criteria for cognitively normal subjects included no history of neurologic or psychiatric disorders, an MMSE score ranging between 27 and 30, and a CDR score of 0. AD patients fulfilled the NINDS-ADRDA criteria and had MMSE scores between 20 and 26 and a CDR score of 0.5 or 1.0.

Cognitive assessment

Global cognition was assessed by MMSE and MOCA scores. MMSE and MOCA scores were selected at eight time points: baseline, 6 months, 12 months, 18 months, 24 months, 30 months, 36 months, 42 months, and 48 months.

Brain MRI data

MR imaging examinations were performed with a Magnetom Trio whole-body 3.0-T MR scanner (Siemens, Erlangen, Germany) with a 12-channel head-matrix coil and identical technical parameters at baseline and at follow-up. All subjects underwent T1- and T2-weighted diffusion-weighted imaging scans and fluid-attenuated inversion recovery (FLAIR) sequence imaging. Sagittal 3D T1W structural images were acquired with the following parameters: repetition time/echo time (TR/TE) = 2300/2.98 ms; time inversion (TI) = 900 ms; field of view (FOV) = 256 × 256 mm; flip angle (FA) = 9; section thickness = 1 mm; and 192 sagittal slices. The major parameters of FLAIR images were as follows: TR/TE = 9000/96ms; FOV = 240 × 240 mm; matrix size = 512 × 512; section thickness = 5 mm.

Normalized volumes of target brain compartments (hippocampal volumes, cortical thickness, and total gray matter volume) were quantified with the FreeSurfer image analysis suite (version 5.3.0), which is documented and freely available for download online at http://surfer.nmr.mgh.harvard.edu/. AD signature cortical thickness was defined by averaging cortical thickness of the entorhinal cortex, inferior temporal lobes, middle temporal lobes, and fusiform gyrus, with a lower value indicating more severe AD pathology [18]. White matter hyperintensity (WMH) was defined as the presence of hyperintensity in the white matter area on FLAIR images. Periventricular hyperintensity (PVH) and deep white matter hyperintensity (DWMH) volumes were quantitatively analyzed by a neurologist using 3D-slicer semiautomated freeware (http://www.slicer.org).

Collection and confirmation of neuronal-derived exosomes **(NDEs)** from the blood

The fasting blood of all participants was drawn at baseline between 6 and 7 A.M. and stored in apolypropylene tube containing EDTA. After drawing, the blood samples were centrifuged at 4000 g for 10 min to obtain the plasma. Specific NDEs were isolated according to our published protocol [19]. In brief, using ExoQuick exosome precipitation solution (EXOQ, EXOQ20A-1, System Biosciences, USA), total exosomes were collected from plasma. NDEs were then isolated by coimmunoprecipitation using a rabbit anti-L1 cell adhesion molecule (L1CAM) antibody (eBiosciences, 13-1719-82, San Diego, CA, USA) and labeled with biotin by the EZ-Link sulfo-NHS-biotin system (Thermo Fisher Scientific, 53117, Waltham, MA, USA).

Western blotting (WB) and transmission electron microscopy (TEM) were performed to confirm the success of exosomal collection according to our previous protocols [18]. The degree of purity was verified by WB with the positive exosomal marker TSG101 (Abcam, Cambridge, MA, USA). L1CAM-positive plasma NDEs were characterized based on shape and size using TEM.

Quantification of NDEs and ELISA

L1CAM-positive exosomal proteins were measured by ELISA kits for human NADH ubiquinone oxidoreductase core subunit S3 (NDUFS3) (Abbexa Ltd., abx381746, Cambridge, UK), human succinate dehydrogenase complex subunit B (SDHB) (Abbexa Ltd., abx383076, Cambridge, UK), and synaptosomal-associated protein 25 (SNAP-25) (RayBiotech, Inc., ELH-SNAP25-1, Norcross, GA, USA). The amount of CD81 protein was measured by ELISA kits (RayBiotech, Inc., ELH-CD81-1, Norcross, GA, USA) to normalize

the relative values for each sample [20]. The neural-derived exosome levels of Aβ42, total tau (T-tau), and tau phosphorylated at threonine 181 (P-T181-tau) were measured by ELISA kits according to our published protocol [19].

Statistical analysis

SPSS (version 22.0) and MedCalc (version 19) statistical software was used for the statistical analysis. Tests for the homogeneity of variances were performed. The Kolmogorov-Smirnov test was also performed to ascertain the normality of the distribution of continuous variables. The statistical significance of differences between means for groups conforming to a normal distribution was determined with Student's unpaired t test or one-way analysis of variance (ANOVA) with Bonferroni's post hoc test. The variables with a nonnormal distribution were compared using the nonparametric Mann-Whitney U test or Kruskal-Wallis test. Categorical variables were compared using the χ^2 test. Spearman's correlation was used to test associations between mitochondrial proteins and other core biomarkers in plasma neuronal-derived exosomes. The relationship between mitochondrial proteins and brain structure (adjusted for age and gender) and cognition (adjusted for age, gender and education) was analyzed by multiple linear regression models. Diagnostic accuracy (the area under the curve (AUC)) for pMCI and AD was obtained for mitochondrial proteins using receiver operating curve (ROC) analyses. The associations of mitochondrial proteins with the conversion from MCI to AD were assessed by calculating hazard ratios (HRs) with 95% confidence intervals (CIs) using Cox proportional hazard regression analysis with adjustment for age and gender. All of the tests were two tailed, and the threshold for statistical significance was $P < 0.05$.

Results

Baseline demographic and biomarker characteristics of the study participants

The demographic features and biomarker characteristics of cognitively normal type 2 diabetic patients, MCI patients, and AD dementia patients are shown in Table 1. The T2DM participants in the study included 41 cognitively normal controls, 97 individuals with MCI (68 individuals with sMCI; 29 individuals with pMCI) and 36 patients with AD dementia. There were no significant differences in age, sex, educational level, HbA1c or vascular risk factors among the groups. Compared with MCI (pMCI and sMCI) and cognitively normal participants, patients with AD dementia had lower MMSE and MoCA scores. For participants with baseline MRI measurements, total hippocampal volume and AD signature cortical thickness were lower in DM patients with pMCI (5.99 ± 0.79 cm^3, 2.47 ± 0.13 mm) than in sMCI patients (6.39 ± 0.71 cm^3, 2.55 ± 0.19 mm). Total hippocampal volume, AD signature cortical thickness, and total gray matter volume in AD patients (5.11 ± 0.84 cm^3, 2.17 ± 0.33 mm, 548.9 ± 24.8 cm^3, respectively) were lower than those in MCI patients (pMCI: 5.99 ± 0.79 cm^3, 2.47 ± 0.13 mm, 607.5 ± 23.3 cm^3, respectively, and sMCI: 6.39 ± 0.71 cm^3, 2.55 ± 0.19 mm, 609.8 ± 22.4 cm^3, respectively) and cognitively normal

participants (7.24 ± 0.73 cm^3, 2.59 ± 0.18 mm, 612.3 ± 27.5 cm^3, respectively). The PVH in MCI (pMCI: 9028 ± 6726 mm^3 and sMCI: 8376 ± 6026 mm^3) and AD patients (11781 ± 8921 mm^3) was higher than that in cognitively normal participants (6119 ± 4464 mm^3). The exosomal concentrations of Aβ42, T-tau, and P-T181-tau in AD patients (4.15 ± 0.60, 207.2 ± 36.5, and 89.4 ± 22.0 pg/ml, respectively) were higher than those in MCI patients (pMCI: 3.78 ± 0.75, 184.7 ± 25.3, 66.8 ± 16.5 pg/ml, respectively, and sMCI: 3.38 ± 0.84, 167.7 ± 30.0, 57.3 ± 13.2 pg/ml, respectively) and cognitively normal participants (3.17 ± 0.77, 163.5 ± 34.1, 55.9 ± 10.2 pg/ml, respectively). Furthermore, the exosomal concentrations of Aβ42, T-tau, and P-T181-tau in pMCI patients were higher than those in sMCI patients and cognitively normal participants. Compared with MCI (pMCI: 530.6 ± 131.5 pg/ml and sMCI: 593.0 ± 130.4 pg/ml) and cognitively normal participants (627.6 ± 152.0 pg/ml), patients with AD dementia (461.4 ± 116.9 pg/ml) had lower exosomal concentrations of SNAP-25. The exosomal concentrations of SNAP-25 in pMCI patients were lower than those in sMCI and cognitively normal participants.

Table 1
Demographic and clinical characteristics of the participants in baseline

	CN (n = 41)	sMCI (n = 68)	pMCI (n = 29)	AD (n = 36)
Age, years	69.8 ± 7.1	71.0 ± 8.0	72.6 ± 7.7	72.0 ± 7.1
Education, years	9.1 ± 4.9	8.1 ± 5.3	8.8 ± 4.5	8.0 ± 4.9
Gender, female (%)	22 (53.6)	41 (60.3)	16 (55.2)	20 (55.6)
Duration of type 2 diabetes, years	9.2 ± 3.6	9.6 ± 3.3	10.8 ± 4.2	11.0 ± 4.1[a]
HbA1c (%)	7.9 ± 1.4	7.7 ± 2.0	7.9 ± 2.2	8.8 ± 3.1
BMI, kg/m^2	24.9 ± 2.4	25.1 ± 3.1	25.0 ± 2.9	25.3 ± 3.0
Hypertension, n (%)	10(24.4)	12(17.6)	6(20.7)	7(19.4)
Hyperlipidemia, n (%)	20(48.8)	31(45.6)	14(48.3)	17(47.2)
Current smoker, n (%)	4(9.8)	5(7.4)	2(6.9)	3(8.3)
Current drinker, n (%)	7(17.1)	11(16.1)	4(13.8)	5(13.9)
MMSE	28.8 ± 1.0	25.4 ± 1.5 [a]	25.3 ± 1.5 [a]	23.2 ± 1.7 [a, b, c]
MoCA	26.8 ± 1.7	21.6 ± 1.9 [a]	21.5 ± 1.6 [a]	18.2 ± 3.4 [a, b, c]
Total hippocampal volume, cm^3	7.24 ± 0.73	6.39 ± 0.71 [a]	5.99 ± 0.79 [a, b]	5.11 ± 0.84 [a, b, c]
AD signature cortical thickness, mm	2.59 ± 0.18	2.55 ± 0.19	2.47 ± 0.13 [a, b]	2.17 ± 0.33 [a, b, c]
Total gray matter volume, cm^3	612.3 ± 27.5	609.8 ± 22.4	607.5 ± 23.3	548.9 ± 24.8 [a, b, c]
PVH, mm^3	6119 ± 4464	8376 ± 6026 [a]	9028 ± 6726 [a]	11781 ± 8921 [a, b]

Abbreviations: CN cognitively normal, sMCI stable mild cognitive impairment, pMCI progressive mild cognitive impairment, AD Alzheimer's disease, BMI body mass index, MMSE Mini Mental State Examination, MoCA Montreal Cognitive Assessment, AD signature cortical thickness: cortical thickness in AD signature regions calculated as the average of cortical thickness in entorhinal, inferior temporal, middle temporal, and fusiform regions, PVH periventricular hyperintensities, DWMH deep white matter hyperintensities, NDEs neuronal-derived exosomes, SNAP-25, Synaptosomal - associated protein 25.

[a] Significant at $P < 0.05$ versus CN; [b] Significant at $P < 0.05$ versus sMCI; [c] Significant at $P < 0.05$; Significant at $P < 0.05$ versus pMCI; [d] Significant at $P < 0.05$ versus AD.

	CN (n = 41)	sMCI (n = 68)	pMCI (n = 29)	AD (n = 36)
DWMH, mm^3	2871 ± 4756	3836 ± 4808	4367 ± 5179	6103 ± 6789
NDEs Aβ42, pg/ml	3.17 ± 0.77	3.38 ± 0.84	3.78 ± 0.75 [a, b]	4.15 ± 0.60 [a, b, c]
NDEs T-tau, pg/ml	163.5 ± 34.1	167.7 ± 30.0	184.7 ± 25.3 [a, b]	207.2 ± 36.5 [a, b, c]
NDEs P-T181-tau, pg/ml	55.9 ± 10.2	57.3 ± 13.2	66.8 ± 16.5 [a, b]	89.4 ± 22.0 [a, b, c]
NDEs SNAP-25, pg/ml	627.6 ± 152.0	593.0 ± 130.4	530.6 ± 131.5 [a, b]	461.4 ± 116.9 [a, b, c]

Abbreviations: CN cognitively normal, sMCI stable mild cognitive impairment, pMCI progressive mild cognitive impairment, AD Alzheimer's disease, BMI body mass index, MMSE Mini Mental State Examination, MoCA Montreal Cognitive Assessment, AD signature cortical thickness: cortical thickness in AD signature regions calculated as the average of cortical thickness in entorhinal, inferior temporal, middle temporal, and fusiform regions, PVH periventricular hyperintensities, DWMH deep white matter hyperintensities, NDEs neuronal-derived exosomes, SNAP-25, Synaptosomal - associated protein 25.

[a] Significant at $P < 0.05$ versus CN; [b] Significant at $P < 0.05$ versus sMCI; [c] Significant at $P < 0.05$; Significant at $P < 0.05$ versus pMCI; [d] Significant at $P < 0.05$ versus AD.

Plasma neuroexosomal mitochondrial protein levels in different diagnostic groups

Plasma neuroexosomal NDUFS3 (Fig. 1A) and SDHB (Fig. 1B) levels were significantly lower in T2DM patients with AD dementia (232.7 ± 63.4, 1360.7 ± 328.5, pg/ml) and pMCI (274.4 ± 78.6, 1536.7 ± 342.8, pg/ml) than in cognitively normal subjects (333.9 ± 96.7, 2050.4 ± 628.9, pg/ml) (P < 0.001 for both groups). Lower neuroexosomal NDUFS3 (Fig. 1A) and SDHB (Fig. 1B) levels were found in AD dementia (232.7 ± 63.4, 1360.7 ± 328.5, pg/ml) than in sMCI (319.9 ± 109.8 pg/ml, $P < 0.001$; 1824.7 ± 606.4 pg/ml, $P < 0.001$) and pMCI (274.4 ± 78.6 pg/ml, $P < 0.05$; 1536.7 ± 342.8 pg/ml, $P < 0.05$). We also found that plasma neuroexosomal NDUFS3 (Fig. 1A) and SDHB (Fig. 1B) levels were lower in pMCI (274.4 ± 78.6 pg/ml, 1536.7 ± 342.8 pg/ml) than in sMCI (319.9 ± 109.8 pg/ml, $P < 0.05$; 1824.7 ± 606.4 pg/ml, $P < 0.05$) subjects.

Associations between mitochondrial proteins and Aβ42 in plasma neuronal-derived exosomes

There were no significant associations between NDUFS3 and SDHB and Aβ42 in plasma neuronal-derived exosomes in CN ($r = -0.195$, $P = 0.222$; $r = -0.259$, $P = 0.102$) or sMCI ($r = -0.103$, $P = 0.405$; $r = -0.225$, $P = 0.065$) subjects (Fig. 2A, Fig. 2B). NDUFS3 and SDHB were negatively correlated with Aβ42 in pMCI

(NDUF: r = - 0.462 P = 0.012; r = - 0.622, P < 0.001) and AD (r = - 0.527, P = 0.001; r = - 0.449, P = 0.006) patients (Fig. 2A, Fig. 2B)

Associations between mitochondrial proteins and tau biomarkers in plasma neuronal-derived exosomes

There were no significant associations between NDUFS3 and T-tau in plasma neuronal-derived exosomes in CN subjects (r = - 0.280, P = 0.076) (Fig. 3A). NDUFS3 was negatively correlated with P-181T-tau in CN subjects (r = - 0.321, P = 0.041) (Fig. 3C). There were no significant associations between SDHB and T-tau (r = - 0.289, P = 0.067) or P-181T-tau (r = - 0.277, P = 0.079) in plasma neuronal-derived exosomes in CN subjects (Fig. 3B, Fig. 3D). NDUFS3 and SDHB were not associated with T-tau in sMCI patients (Fig. 3A, Fig. 3B). There were significant associations of NDUFS3 and SDHB with P-181T-tau in plasma neuronal-derived exosomes in sMCI subjects (r = - 0.271, P = 0.026; r = - 0.276, P = 0.022) (Fig. 3C, Fig. 3D). NDUFS3 and SDHB were negatively correlated with T-tau (r = - 0.475, P = 0.009; r = - 0.448, P = 0.015) (Fig. 3A, Fig. 3B) and P-181T-tau (r = - 0.579, P = 0.001; r = - 0.448, P = 0.015) (Fig. 3C, Fig. 3D) in plasma neuronal-derived exosomes in pMCI patients. There were also significant associations of NDUFS3 and SDHB with T-tau (r = - 0.492, P = 0.002; r = - 0.583, P < 0.001) (Fig. 3A, Fig. 3B) and P-181T-tau (r = - 0.589, P < 0.001; r = - 0.699, P < 0.001) (Fig. 3C, Fig. 3D) in plasma neuronal-derived exosomes in AD patients.

Associations between mitochondrial proteins and SNAP-25 in plasma neuronal-derived exosomes

There were no significant associations between NDUFS3 and SDHB and SNAP-25 in plasma neuronal-derived exosomes in CN (r = 0.126, P = 0.433; r = 0.218, P = 0.170) (Fig. 4A, Fig. 4B) and sMCI (r = 0.205, P= 0.094; r = 0.214, P = 0.080) (Fig. 4A, Fig. 4B) subjects. Both NDUFS3 and SDHB were positively correlated with SNAP-25 in plasma neuronal-derived exosomes in pMCI (r = 0.614, P < 0.001; r = 0.633, P < 0.001) (Fig. 4A, Fig. 4B) and AD (r = 0.547, P = 0.001; r = 0.623, P < 0.001) (Fig. 4A, Fig. 4B) patients.

Plasma neuroexosomal mitochondrial proteins in relation to brain structure and WMH

The associations of mitochondrial proteins with baseline brain structure and WMH are shown in Table 2. Both NDUFS3 and SDHB were correlated with total hippocampal volumes in pMCI (β = 0.597, P = 0.011; β = 0.507, P = 0.028) and AD (β = 0.531, P = 0.001; β = 0.464, P = 0.004) patients. There were also significant associations of NDUFS3 and SDHB with AD signature cortical thickness in pMCI (β = 0.465, P = 0.014; β = 0.466, P = 0.010) and AD (β = 0.475, P = 0.003; β = 0.465, P = 0.003) patients. NDUFS3 and SDHB were only correlated with total gray matter volume in the AD group (β = 0.445, P = 0.005; β = 0.365, P = 0.025). Except for weak associations of NDUFS3 and SDHB with baseline PVH volumes in the AD group, neither baseline NDUFS3 nor SDHB levels were correlated with WMH (PVH and DWMH) volumes in the other groups.

Table 2
Analysis of association between mitochondrial proteins and baseline brain structure and WMH

	Total hippocampal volumes		AD signature cortical thickness		Total gray matter volume		PVH		DWMH	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
CN										
NDUFS3	0.150	0.356	-0.049	0.761	0.008	0.963	-0.222	0.159	-0.095	0.554
SDHB	0.143	0.380	-0.158	0.323	-0.101	0.541	-0.226	0.151	-0.156	0.327
sMCI										
NDUFS3	0.122	0.326	0.201	0.102	0.066	0.592	-0.176	0.159	-0.150	0.232
SDHB	0.259	0.082	0.168	0.178	-0.015	0.907	-0.142	0.254	-0.130	0.299
pMCI										
NDUFS3	0.597	0.011	0.465	0.014	0.353	0.065	-0.122	0.549	-0.143	0.469
SDHB	0.507	0.028	0.466	0.010	0.344	0.062	-0.135	0.490	-0.242	0.199
AD										
NDUFS3	0.531	0.001	0.475	0.003	0.445	0.005	-0.323	0.046	-0.293	0.080
SDHB	0.464	0.004	0.465	0.003	0.365	0.025	-0.321	0.055	-0.277	0.110

Abbreviations: CN cognitively normal, sMCI stable mild cognitive impairment, pMCI progressive mild cognitive impairment, AD Alzheimer's disease, NDUFS3 NADH ubiquinone oxidoreductase core subunit S3, SDHB Succinate dehydrogenase complex subunit B, AD signature cortical thickness: cortical thickness in AD signature regions calculated as the average of cortical thickness in entorhinal, inferior temporal, middle temporal, and fusiform regions, PVH periventricular hyperintensities, DWMH deep white matter hyperintensities.

During the follow-up period, 29 MCI patients progressed to developing AD and were further subjected to brain MRI at the point of dementia conversion. Analysis of the correlation between mitochondrial proteins and changes in brain structure or WMH was performed. Lower baseline NDUFS3 and SDHB levels were correlated with volumetric loss in the total hippocampus (β = - 0.497, *P* = 0.007; β = - 0.524, *P* = 0.002), total gray matter (β = - 0.494, *P* = 0.008; β = - 0.431, *P* = 0.017), and reduced AD signature cortical thickness (β = - 0.302, *P* = 0.114; β = - 0.294, *P* = 0.111). There were no significant associations between baseline NDUFS3 or SDHB and increased PVH or DWMH volumes (data not listed).

Diagnostic power of plasma neuroexosomal mitochondrial proteins for pMCI and AD

The results obtained from the ROC curve analyses of the pMCI patients and CN subjects revealed that biomarkers in plasma neuronal-derived exosomes had lower diagnostic value for patients with pMCI

(Table 3). Compared with Aβ42, T-tau, and SNAP-25, NDUFS3 and SDHB had almost the same range of diagnostic accuracy for AD (NDUFS3 vs. Aβ42, *P* = 0.594; NDUFS3 vs. T-tau, *P* = 0.862; NDUFS3 vs. SNAP-25, *P* = 0.724; SDHB vs. Aβ42, *P* = 0.975; SDHB vs. T-tau, *P* = 0.468; SDHB vs. SNAP-25, *P* = 0.340) (Fig. 5). Compared with P-181T-tau, SDHB also had almost the same range of diagnostic accuracy for AD (*P* = 0.114). However, P-181T-tau provided higher diagnostic accuracy than NDUFS3 for AD (*P* = 0.029) (Fig. 5).

Table 3
AUC of biomarkers in plasma neuronal-derived exosomes

	Aβ42	T-tau	P-181T-tau	SNAP-25	NDUFS3	SDHB
pMCI	0.707(0.585-0.829)	0.697(0.554-0.803)	0.714(0.592-0.836)	0.678(0.544-0.802)	0.680(0.555-0.805)	0.746(0.633-0.859)
	(*P* = 0.003)	(*P* = 0.011)	(*P* = 0.002)	(*P* = 0.012)	(*P* = 0.011)	(*P* < 0.001)
AD	0831(0.742-0.920)	0.790(0.691-0.889)	0.907(0.839-0.975)	0.779(0.678-0.880)	0.801(0.705-0.896)	0.833(0.745-0.920)
	(*P* < 0.001)	(*P* < 0.001)	(*P* < 0.001)	(*P* < 0.001)	(*P* < 0.001)	(*P* < 0.001)

Plasma neuroexosomal mitochondrial proteins in relation to cognition and future cognitive changes

There were no significant associations of NDUFS3 and SDHB in plasma neuronal-derived exosomes with baseline MoCA scores in CN (β = 0.072, *P* = 0.667; β = 0.143, *P* = 0.395) and sMCI (β = 0.135, *P* = 0.269; β = 0.203, *P* = 0.092) subjects. SDHB levels were correlated with baseline MoCA scores in pMCI (β = 0.448, *P* = 0.037) patients. There were no significant associations between NDUFS3 and baseline MoCA scores in pMCI (β = 0.260, *P* = 0.199) patients. Both NDUFS3 and SDHB were associated with baseline MoCA scores in AD patients (β = 0.431, *P* = 0.019; β = 0.418, *P* = 0.016).

Low NDUFS3 and SDHB levels in plasma neuronal-derived exosomes were correlated with a more rapid decrease in MoCA scores in pMCI during the clinical follow-up period (β = 0.481, *P* = 0.041; β = 0.474, *P* = 0.034).

Plasma neuroexosomal mitochondrial proteins predict conversion from MCI to AD

We analyzed whether plasma neuroexosomal mitochondrial proteins predicted conversion from MCI to AD. Cox proportional hazard regression analysis was performed for NDUFS3 and SDHB as continuous variables after adjusting for age and sex. Only SDHB significantly predicted conversion from MCI to AD. The hazard ratio (HR) was then calculated for SDHB as a dichotomous variable using the median values of SDHB as a cutoff (adjusting for age and sex). Subjects with low SDHB (HR 0.387, *P* = 0.018), corresponding to subjects whose SDHB values were ≤ 1645.8 pg/ml, progressed much more rapidly to

AD than subjects with higher values (> 1645.8 pg/ml, corresponding to the higher median values of SDHB) (Fig. 6).

Discussion

In the present diabetic longitudinal study, we investigated the associations of mitochondrial proteins (NDUFS3 and SDHB) in plasma neuronal-derived exosomes with other key biomarkers across the AD spectrum. Additional evidence in the results showed that mitochondrial proteins reflect the AD pathophysiologic process and are able to distinguish between diagnostic groups. Finally, low mitochondrial protein levels were predictive of clinical conversion from MCI to AD and brain structure injury.

Mitochondrial dysfunction is a key feature of both diabetes mellitus and neurodegenerative diseases, including AD. Mitochondrial dysfunction and oxidative stress have been extensively reported in patients with diabetes and AD, as well as in rodent models of all of these conditions. For example, a large decrease in the NDUFS3 protein subunit of complex I decreased the level of the mRNA and impaired the catalytic activity of the complex in diabetic rats compared to controls [21]. In addition, the activity of succinate dehydrogenase (complex II), a key marker of mitochondrial content, was reduced by 10-30% in diabetic vs. control mice [22]. In rat primary cortical neurons, high glucose concentrations cause decreased mitochondrial respiration, protein expression of peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC1α) and complex I of the electron transport chain, and insulin resistance[23]. Impairment of the respiratory chain has also been observed in mitochondria isolated from sucrose-treated wild-type and AD transgenic mice [24]. Some studies have found that mitochondrial complexes, including subunits of the electron transport chain (including NDUFS3 and SDHB) and ATP synthase, were altered in AD patients [25–27]. Furthermore, mitochondrial dysfunction in the nervous system is considered a key pathophysiologic feature of early-stage AD[28, 29] and could be a promising biomarker for early AD by measuring mitochondrial function [30]. In the present study, we show that, comparing CN and sMCI diabetic participants, the protein cargo of NDEs in pMCI and AD patients was characterized by lower levels of the mitochondrial components NDUFS3 (complex I) and SDHB (complex II). SDHB in neuronal-derived exosomes offers predictive value for future disease progression in MCI subjects. This finding suggests that neuroexosomal mitochondrial proteins (NDUFS3 and SDHB) are an early pathophysiological indicator of AD-related mitochondrial dysfunction.

In the diabetic brain, both Aβ and tau can cause mitochondrial alterations leading to neuronal energy deficits, synaptic disturbances and neurodegeneration[10]. In our study, there were significant correlations of the levels of mitochondrial proteins (NDUFS3 and SDHB) with Aβ42, tau, and SNAP-25 (markers indicating synaptic damage) in plasma neuronal-derived exosomes in pMCI and AD patients. Hyperglycemia exacerbated mitochondrial defects, synaptic injury, and cognitive dysfunction in the brains of transgenic AD mice overexpressing amyloid-β, as shown by decreased mitochondrial respiratory complex I enzyme activity and a greatly decreased mitochondrial respiratory rate [31]. Aβ has toxic effects on mitochondrial respiration, synthesis of ATP, and the activities of various enzymes related to

energy production, including the I and II enzyme complexes in the mitochondrial respiratory chain [32]. Overexpression and hyperphosphorylation of tau appear to impair mitochondrial axonal transportation, mitochondrial dynamics and function, and finally neuronal health [33]. Moreover, mitochondrial dysfunction is also involved in promoting tau pathology in AD [34, 35]. Mitochondria are essential for synaptic function by providing energy and regulating intrasynaptic metabolic homeostasis [36, 37]. Several years of research have shown that synaptic pathology and mitochondrial oxidative damage, caused by amyloid beta and phosphorylated tau, are early events in AD progression [38].

A recent multicenter study showed that the levels of Aβ42 and tau biomarkers in neuronal-derived exosomes were highly correlated with their levels in cerebrospinal fluid and confirmed that plasma neuroexosomal Aβ42, T-tau, and P-T181-tau have the same capacity as those in cerebrospinal fluid for the diagnosis of AD [5]. In the present study, our results showed that mitochondrial proteins (NDUFS3 and SDHB) offer diagnostic sensitivity for AD that is comparable to that of Aβ42, T-tau, and P-T181-tau in plasma neuronal-derived exosomes. In addition, we found associations between low neuroexosomal mitochondrial protein levels and volumetric loss in the total hippocampus and total gray matter, as well as reduced AD signature cortical thickness in pMCI. These findings therefore suggest that plasma neuroexosomal mitochondrial proteins might be potential diagnostic biomarkers for early-stage AD.

Some limitations of our study should be considered when interpreting the results. First, the results were drawn from a small-scale hospital-based study, and future investigations are necessary to replicate and validate our findings in a large population of patients. Second, we did not have additional information, such as CSF data and pathological evidence or inflammatory biomolecules, to confirm the results. Third, our results are limited to a cohort of elderly individuals with type 2 diabetes; therefore, they cannot be generalized to the general population.

Conclusion

In summary, we identified significantly decreased plasma neuroexosomal mitochondrial proteins in the predemential stages of AD in diabetic participants, and lower concentrations were correlated with higher hippocampal and gray matter atrophy, reduced AD signature cortical thickness, and a reduced rate of cognitive decline in some stages of AD. These findings support the use of neuroexosomal mitochondrial proteins as potential diagnostic biomarkers in AD.

Abbreviations

AD: Alzheimer's disease; pMCI: progressive mild cognitive impairment; sMCI: stable mild cognitive impairment; NDEs: neuronal-derived exosomes; NDUFS3: NADH ubiquinone oxidoreductase core subunit S3; SDHB: succinate dehydrogenase complex subunit B; WMH: White matter hyperintensity; PVH: Periventricular hyperintensity; DWMH: deep white matter hyperintensity; Aβ: amyloid-β; T-tau: total tau; P-T181-tau: tau phosphorylated at threonine 181.

Declarations

Acknowledgements

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Authors' contributions

JBZ conceived and designed the study. HYC, RY, CS, BL, TW and SKZ performed experiments. MFL performed neuroimaging analyses. TQS, HRS and JBZ analyzed and interpreted data. HYC and RY drafted the manuscript. JBZ and ZGL revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The ethics committee of Weihai Municipal Hospital approved this study. Each participant gave an informed written consent before enrollment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

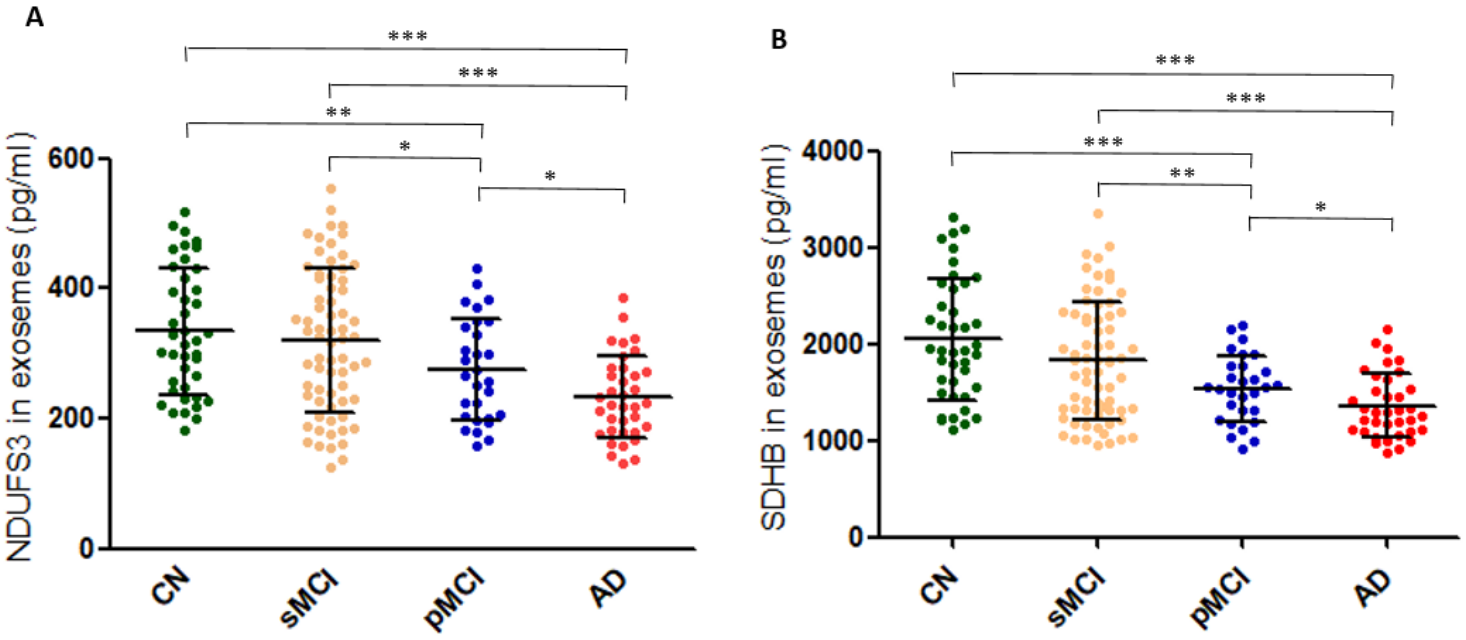

Figure 1

Plasma neuronal-derived exosomes NDUFS3 and SDHB levels in different diagnostic groups. Scatter plots showing plasma neuronal-derived exosomes NDUFS3 (A) and SDHB (B) levels in T2DM subjects

with cognitively normal (CN), stable MCI (sMCI), progressive MCI (pMCI) and Alzheimer's disease (AD). ***P < 0.001, **P < 0.01, *P < 0.05.

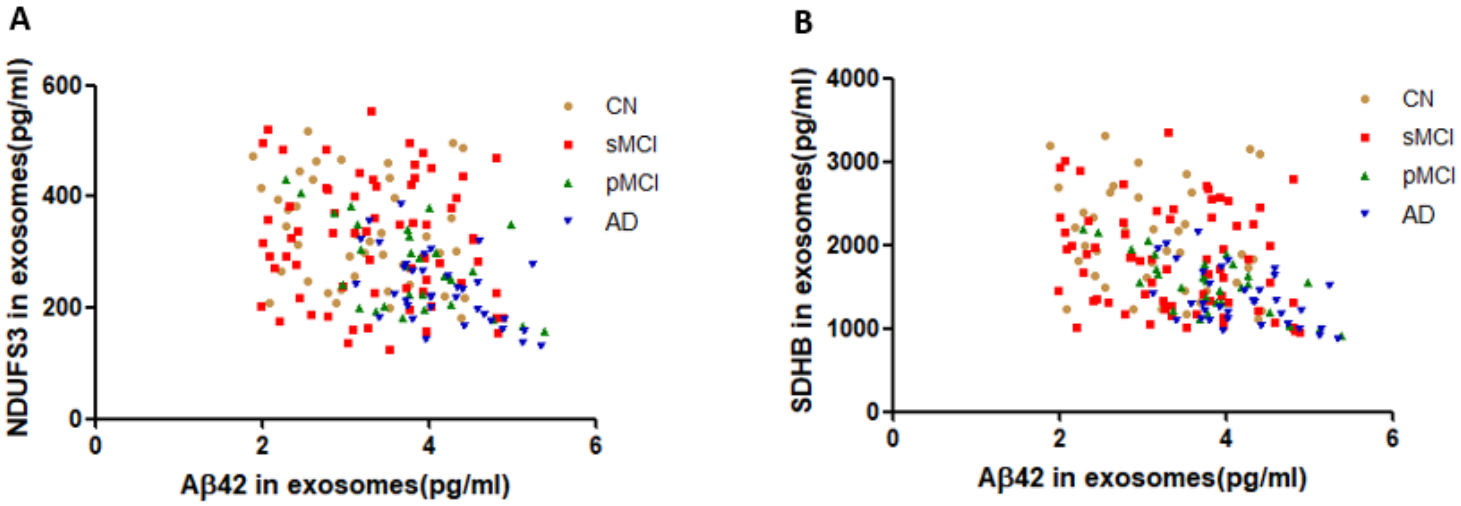

Figure 2

Mitochondrial proteins levels in relation to Aβ42 in plasma neuronal-derived exosomes. Correlations between NDUFS3 (A) and SDHB (B) levels and Aβ42 in different diagnostic groups.

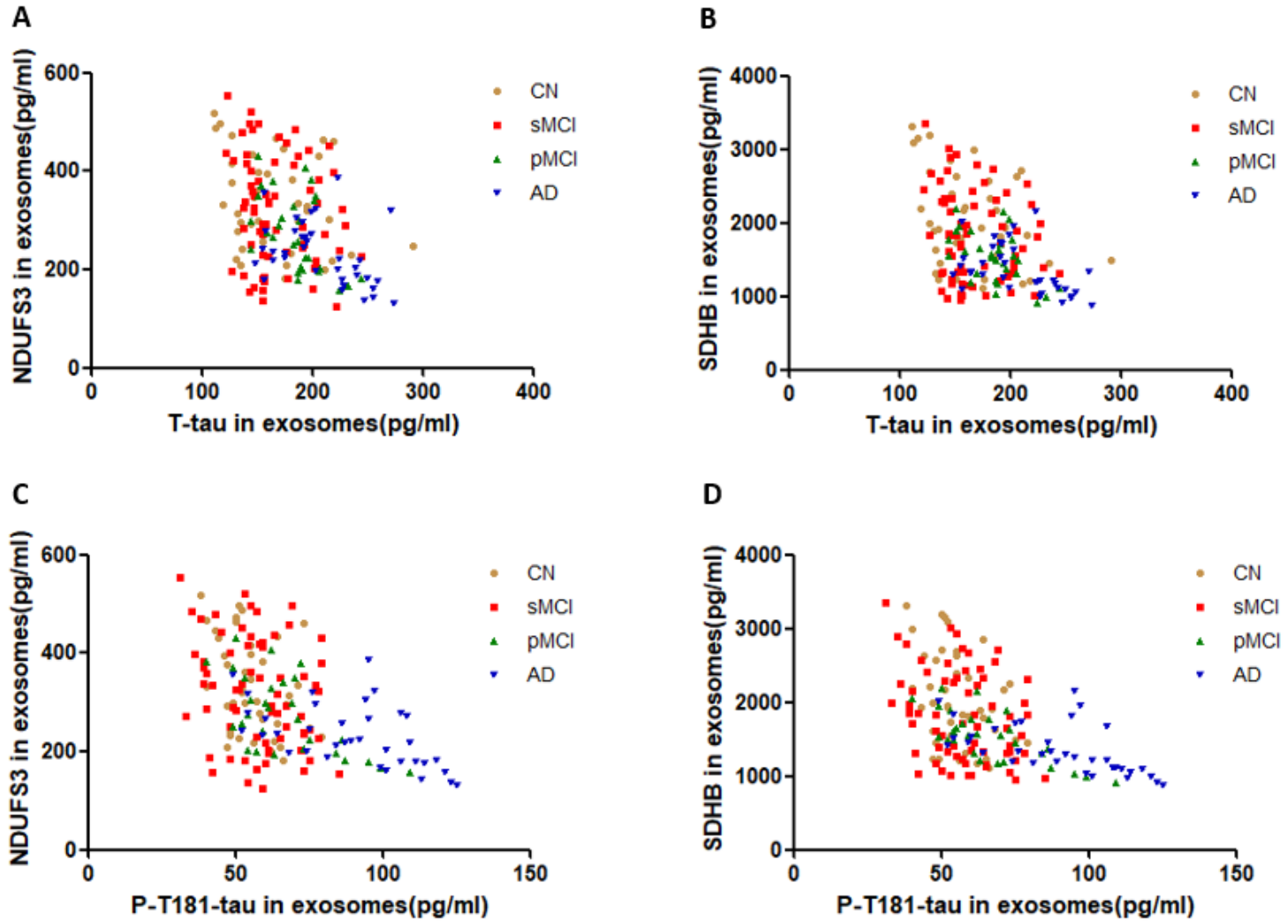

Figure 3

Mitochondrial proteins levels in relation to tau biomarkers in plasma neuronal-derived exosomes. Correlations between NDUFS3 (A) and SDHB (B) levels and T-tau in different diagnostic groups. Correlations between NDUFS3 (C) and SDHB (D) levels and T-tau in different diagnostic groups.

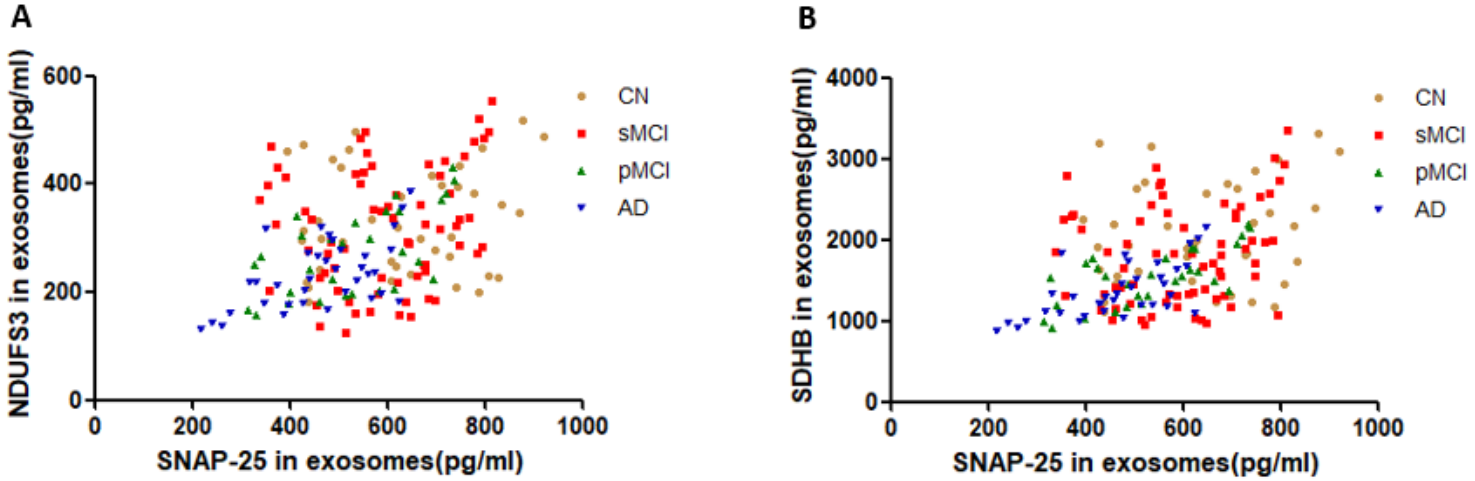

Figure 4

Mitochondrial proteins levels in relation to SNAP-25 in plasma neuronal-derived exosomes. Correlations between NDUFS3 (A) and SDHB (B) levels and SNAP-25 in different diagnostic groups.

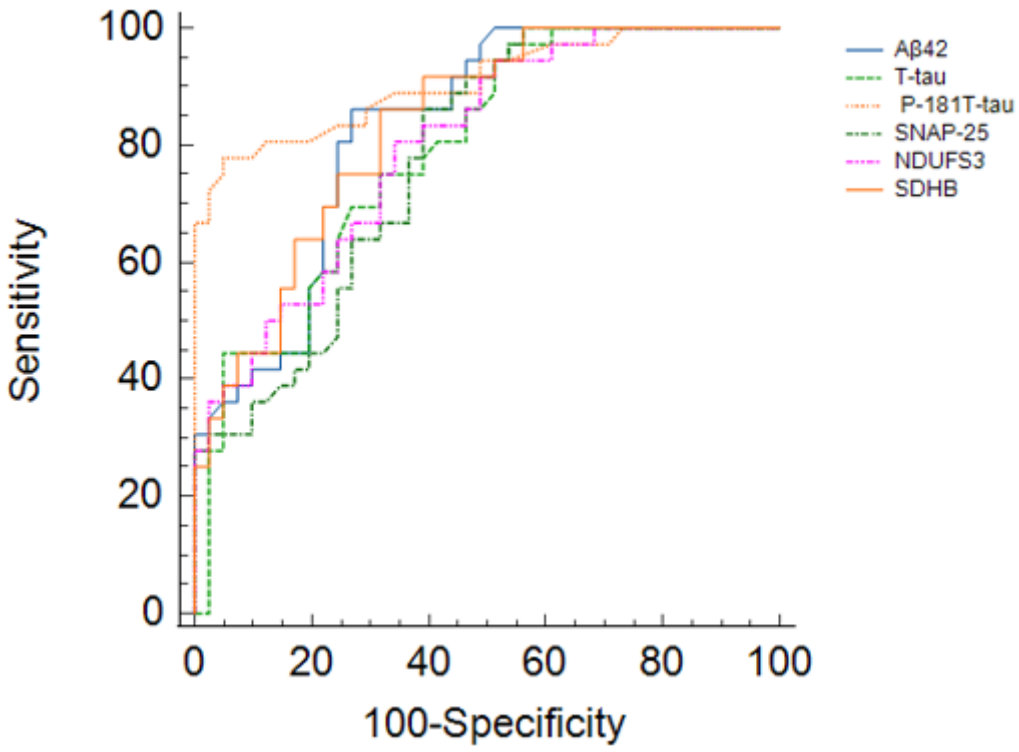

Figure 5

ROC analyses. ROC analyses were performed to test the plasma neuro-exosomal mitochondrial proteins in relation to clinical diagnoses for AD.

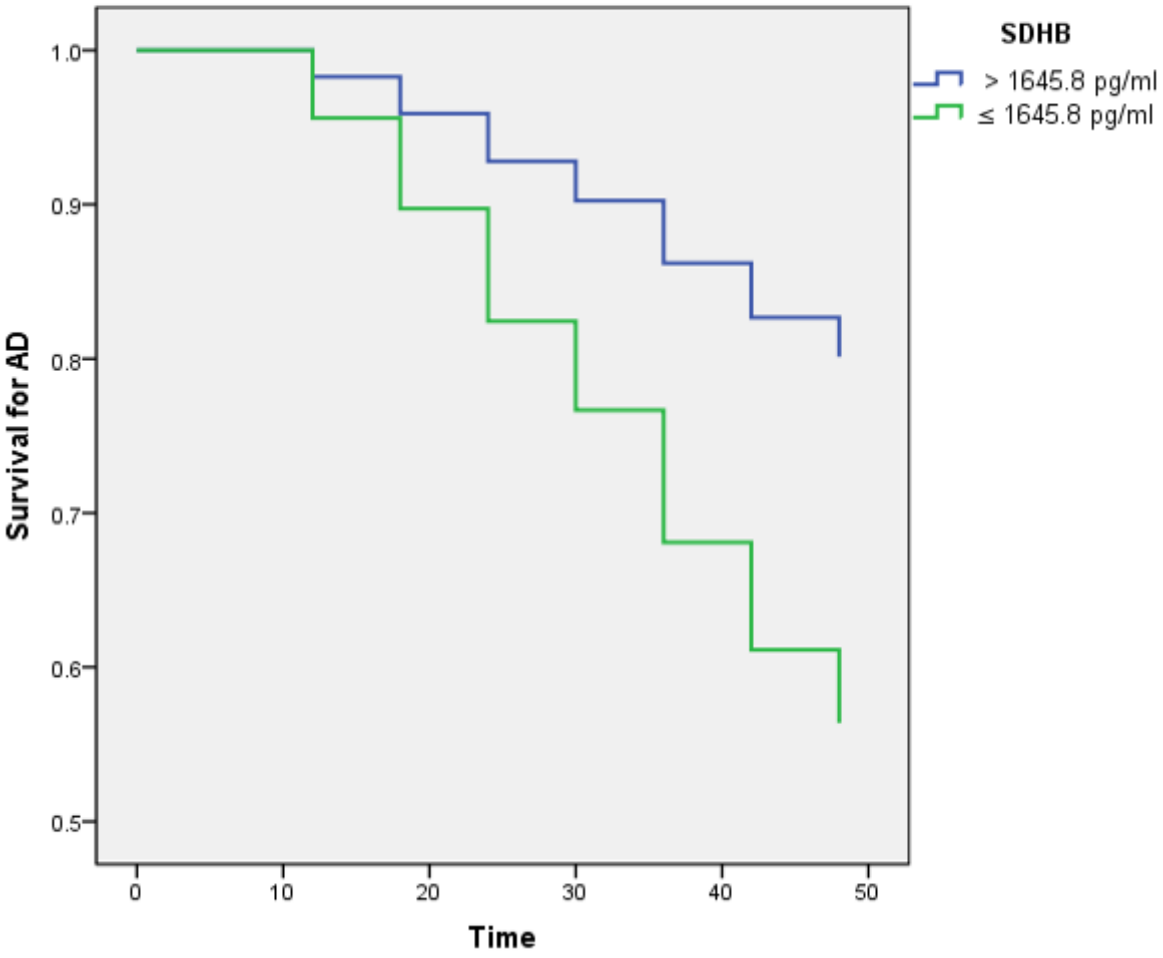

Figure 6

Baseline SDHB in plasma neuronal-derived exosomes as predictors of conversion from MCI to AD. Survival from AD as a function of SDHB in plasma neuronal-derived exosomes measures (dichotomized at the median values) are shown. Analyses were adjusted for age and gender. Cutoff value was 1645.8 pg/ml for SDHB.