Elevated neurofilament light chain in plasma is associated with reduced right hippocampal and amygdala volume in Alzheimer's patients

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Abstract

Background Plasma neurofilament light (NfL) levels have been considered as an especially promising biomarker for dementia, however, the mechanism of NfL regulating cognition is not very clear. Methods 43 amnesic mild cognitive impairment(aMCI), 35 Alzheimer's disease (AD) and 30 cognitively normal subjects were recruited. Plasma NfL levels were examined by the Single Molecule array (Simoa) technique; the volumes of the hippocampus and amygdala were calculated and compared by T1-weighted MRI; and cognitive function was assessed by the Beijing version of the Montreal Cognitive Assessment (MoCA) Results Our results showed significantly increased plasma NfL levels in AD group (29.42 pg/ml) compared to aMCI(15.92 pg/ml) group and normal (12.85 pg/ml) group (both $p < 0.001$), while there was no statistical difference ($p>0.05$) between aMCI group and normal group. And the results of partial correlation analysis showed that plasma NfL levels were negatively correlated ($p<0.05$)with MoCA total score ($r=-0.415$, $p=0.013$), right hippocampal volume($r=-0.335$,$p=0.036$) and right amygdala volume($r=-0.337$, $p=0.048$). Conclusions NfL in plasma of AD patients is significantly increased, and the protein is related to atrophy of right hippocampus and right amygdala.

1. Introduction

Neurofilament light chain (NfL) plays an important role in axon transmission and function maintenance, and is the most abundant intermediate filament protein in myelinated subcortical axons [1]. Previous studies pointed that NFL is an ideal marker of large-caliber axonal degeneration, and increased NfL levels incerebrospinal fluid(CSF) are like to reflect neurodegeneration-related axonal injury[2], such as Alzheimer's disease (AD), frontotemporal lobar degeneration (FTLD), multiple sclerosis and amyotrophic lateral sclerosis [3,4]. In addition, CSF NfL levels have also been proved to be an effective way to distingusih FTLD from early-onset AD given that NfL levels in AD are lower in early onset compared to those in late onset presentations[5,6]. However, NfL in cerebrospinal fluid is not applicable and difficult to accept for many elderly people, Therefore, blood-based measurement of NfL might be more desirable, since the collection of blood sample is relatively less invasive and more applicable[7].

So far, there have been a few studies on NfL expression patterns in the blood of AD patients in China. For example, Liu S [8]et al found that gastric cancer subjects expressed lower plasma NfL levels but AD subjects expressed higher plasma NfL levels than normal controls. Hu H[9] et al found that plasma NfL concentration and its rate of change had already increased abnormally in the preclinical phase of AD. And Lin YS[10] et al also found that plasma NfL was significantly increased in the AD group, compared with the control, mild cognitive impairment (MCI), non-demented Parkinson's disease (PD), and Parkinson's disease dementia groups. These conclusions suggest NFL in plasma may represent a biomarker of cognitive decline in AD, and it is possible to mark the onset of neurodegeneration in subjects at risk for AD familial disease[1]. However, the mechanism of NfL regulating cognition is not very clear.

Neuroimaging using Magnetic Resonance Imaging (MRI) has been widely used to describe the atrophy pattern of cognitive related brain regions in AD and FTLD as well as to find differential trajectories along the different disease stages[11–13]. And structural MRI of medial temporal atrophy (MTA) is considered to be a biomarker for an early diagnosis of MCI and AD[14,15], specifically speaking, volume reduction of medial temporal lobe, including amygdala and hippocampus has been proved to be an early manifestation of AD[16]. The relationship between classical AD biochemical markers AD and neuroimaging features and their reciprocal influence have been studied during both the preclinical phases and clinical of the disease[3]. For example,Mattsson N[17] et al pointed out that high plasma NfL levels in the MCI and AD cohort were associated with smaller hippocampal volumes, thinner cortices and larger ventricular. However, it is not known whether the above conclusions are applicable to Chinese people.

Therefore, in this context, our goals were (a) to provide a descriptive analysis of plasma NfL levels and structural patterns (amygdala volume and hippocampal volume) in early-onset AD, amnestic mild cognitive impairment (aMCI) and normal control(NC); (b) to study the relationship between early-onset AD, aMCI and NC brain structural measures/cognitive function and plasma NfL levels.

2. Materials And Methods

2.1 Data base

Data were obtained from the China Longitudinal Aging Study (CLAS)[18] database, which was launched in 2013 as a large scale longitudinal cohort study in China and was led by principal investigator Shifu Xiao, MD and PhD. The CLAS participants were recruited from 20 target communities (ie, 2 rural and 18 urban) located in the eastern, mid, and western parts of China, and all of them were permanent residents aged 60 years or older[19].

2.2 Participants

Our study enrolled 30 normal controls (NC), 43 aMCI subjects and 35AD subjects, who were matched in age, gender and education. All subjects diagnosed with AD fulfilled the following criteria: (a) age ≥ 60 years; (b) met the National Institute on Aging-Alzheimer's Association (NIA-AA) 2011 criteria for dementia and the DSM-V 2013 criteria[20]; (c) global Clinical Dementia Rating (CDR) scores between 0.5 and 2; (d) Mini-Mental State Examination (MMSE) scores below 24. All subjects diagnosed with aMCI fulfilled the following criteria[21]:(a) age ≥ 60 years ; (b)memory complaints;(c) objective memory impairment(Mini-Mental State Examination (MMSE) scores below 25; (d) normal general cognitive function; (e)intact activities of daily living; and (f) absence of dementia: CDR scores of 0.5. The NCs fulfilled the following criteria: (a) age ≥ 60 years; (b) no reports of experiencing memory loss; (c) CDR scores of 0 and MMSE scores between 25 and 30; and (d) absence of serious mental and physical diseases.

2.3 General demographic data

General demographic data, such as age, gender, education, smoking history, drinking history, tea drinking history and disease history(diabetes and hypertension) were collected with standardized questionnaires, and in the form of self-report.

2.4 Plasma NfL

Plasma NFL was examined by a highly sensitive assay on the Single Molecule Array (Simoa™) platform[22]. SiMoA NfL assay advantage kits (product #103186) were commercially obtained from Quanterix corporation(MA, USA). Critical reagents including buffer quality controls and recombinant human NfL standards were supplied frozen for single use only[23]. And all the samples were measured in duplicate.

2.5 Neuropsychological Tests

Montreal Cognitive Assessment-Chinese Version (MoCA-CV)[24] was used to assess the overall cognitive function of all subjects. The MOCA is an evaluation tool used to evaluate MCI, developed by Nasreddine [25]et al. And it is able to differentiate between MCI and early dementia as well as between normal and MCI[26]. The MoCA cut-offs (–1 to –2 standard deviations) for cognitive impairment was ranged from <25 to <21 for the lowest educated and <26 to <24 for the highest educated, depending on different age groups[27].

2.6 Magnetic Resonance Imaging

MR images were scanned by using Siemens Magnetom Verio 3.0T scanner (Siemens, Munich, Germany). T1-weighted images were obtained from 176 sagittal slices using 3D magnetization prepared rapid gradient echo acquisition sequence with the following parameters: Spatial resolution = 1∗1∗1.2 mm3, TE = 2.98 ms ,TR = 2300 ms, Flip angle = 9°[28]. The MRI FLAIR data acquisition setting used the following parameters: matrix 256 × 192, FOV = 24 cm ,NEX = 1, TE = 140, InVTime = 2200, TR = 8600[29].

Automated procedures were utilized to ascertain volumetric data. The automated assessment was described by using the Learning Embedding for Atlas Propagation (LEAP) algorithm[30]. The volume and asymmetry with hippocampus and amygdala as well as the brain size index of each subject were extracted.

3. Statistical Analyses

Continuous variables were expressed as mean ± SD and categorical variables were expressed as frequencies (%). Single sample Kolmogorov-Smirnov test was used to check whether the data conform to the normal distribution. Single factor ANOVA was used to compare the data of normal distribution among AD group, aMCI group and normal group, while Kruskal-Wallis H(K) test was used to compare the data of non -normal distribution. And chi square test was used to categorical variables among the three groups. Then partial correlation analysis(controlled for hypertension) was used to explore the association

between plasma NfL and volume of cognitive related brain areas(hippocampus and amygdala). Two-tailed tests were utilized at a significance level of $P < 0.05$, and all statistical analyses were performed using SPSS 22.0 (IBM Corporation, Armonk, NY, USA).

4. Results

There were statistical differences ($p<0.05$) in hypertenion, MoCA, left hippocampus, right hippocampus, left amygdala and right amygdala among AD group, aMCI group and normal group, but no significant differences ($p>0.05$) in age, education, gender, smoker, drinker, tea drinker and diabetes. Table 1 shows the results. Then further comparison showed that the MOCA scores of normal group, aMCI group and AD group decreased in turn. The volume of hippocampus and amygdala and in aMCI group and normal group were larger ($p<0.05$)than that in AD group, but there was no significant difference($p>0.05$) between aMCI group and normal group. However, the concentration of plasma NfL in aMCI group and normal group were lower ($p<0.05$)than that in AD group, but there was no significant difference($p>0.05$) between aMCI group and normal group(figure 1). And table 2 presents the results. Then by using partial correlation analysis and controlled of hypertension, we found that plasma NfL was negatively correlated ($p<0.05$)with MoCA total score ($r=-0.415$, $p=0.013$), right hippocampal volume($r=-0.335$,$p=0.036$) and right amygdala volume($r=-0.337$, $p=0.048$).

5. Discussion

In the present study, we analyzed and compared the plasma concentration of NfL as well as the volume differences in hippocampus and amygdala among patients with AD, patients with aMCI and normal elderly. Finally, we reached some interesting conclusions: a) the concentration of plasma NfL in aMCI group and normal group were lower ($p < 0.05$)than that in AD group, but there was no significant difference($p > 0.05$) between aMCI group and normal group; b) the volume(both left and right) of hippocampus and amygdala in aMCI group and normal group were larger ($p < 0.05$)than that in AD group, but there was no significant difference($p > 0.05$) between aMCI group and normal group; c) plasma NfL was negatively correlated ($p < 0.05$)with MoCA total score ($r=-0.415$, $p = 0.013$), right hippocampal volume($r=-0.335$,$p = 0.036$) and right amygdala volume($r=-0.337$, $p = 0.048$).

NfL is an important cytoarchitectural protein present primarily in large-caliber myelinated axons[31],and increased NfL in CSF will indicate damage or degeneration of these axons. This protein appears to be relatively independent of tau and amyloid levels, and might correlate with symptomology, progression, and survival[32]. Now NfL has been considered as an especially promising biomarker for neurodegeneration because it can be measurable in plasma[33]. Single molecule array (SiMoA) digital immunoassay platform can offer heightened sensitivity by miniaturizing the reaction volume[34], and several biomarkers indicative of peptide fragmentation and neuronal dysfunction can be accurately quantified in peripheral blood. In our study, we used this Simoa technology to compare the plasma NFL concentrations of AD, aMCI and normal elderly people(their gender, age and education are matched), and

found the concentration of plasma NfL in AD group were significantly higher($p < 0.05$) than that in aMCI group and normal group. Previous studies had confirmed that AD was associated with elevated NFL in plasma, so our findings were consistent[8,9]. However, we did not find any difference in plasma NfL between patients with aMCI and normal elderly, which was inconsistent with the conclusion of Zhow W[7] et al. So the relationship between NfL and aMCI needs to be further verified.

Atrophy of the medial temporal lobe, a critical region involved in memory formation, is considered as a recognized marker for AD[35]. The hippocampus and amygdala, both residing in the medial temporal lobe, are proved to be associated with declarative memory and emotional processing, respectively[36]. As the atrophy of hippocampal and amygdalar are already evident in the prodromal stage of AD[37], the volumetric measurements of hippocampus and amygdala have been used to assist the clinical diagnosis of AD[38] and to predict the cognitive status of the elderly[39]. As expected, the volume of hippocampus and amygdala in AD patients was significantly smaller than that in aMCI and normal old people, in concordance with previous publications[39–41]. Then by using partial correlation analysis (adjusting hypertension), we found plasma NfL was negatively correlated with MoCA total score ($r=-0.415$, $p = 0.013$), right hippocampal volume($r=-0.335$,$p = 0.036$) and right amygdala volume($r=-0.337$, $p = 0.048$).

Parbo P[42] et al pointed that plasma NfL levels were inverse correlated with levels of inflammation in cortical areas in AD. Mattsson N[33] et al found that faster increase in plasma NfL levels correlated with faster rates of atrophy and hypometabolism, and faster worsening in global cognition in aMCI and AD. And Weston PSJ[43] et al also found that serum NfL correlated with baseline brain volume and whole-brain atrophy rate in patients with familial Alzheimer disease. So we had jointly confirmed that there was a certain correlation between plasma NfL and cognitive related brain regions. However, we only found that plasma NfL was related to the right hippocampus and right amygdala, but not to the left. Wang L[44] et al found that the functional connectivity between the right hippocampus and a set of regions(such as medial prefrontal cortex, right inferotemporal cortex, right cuneus extending into precuneus, ventral anterior cingulate cortex, left cuneus, right superior and middle temporal gyrus and posterior cingulate cortex)was disrupted in AD. Sohn WS[45] et al also believed that the right hippocampal connectivity was relatively by AD progression. And López-Jaramillo C[46] et also found that lithium-treated bipolar I disorder patients had larger right thalamus than unmedicated patients and controls. Therefore, we speculated that plasma NFL had some intrinsic relationship with the right hippocampus and the right amygdala, although the mechanism was not fully understood.

Our study has certain limitations: first, it's just a cross-sectional study that could not establish a causal link; second, the relatively small sample size reduced the reliability of the study; third, we lacked the gold standard to diagnose AD and aMCI.

6. Conclusions

NFL in plasma of AD patients was significantly increased, and the protein was related to atrophy of right hippocampus and right amygdala.

7. Abbreviations

NfL	Plasma neurofilament light
aMCI	amnesic mild cognitive impairment
AD	Alzheimer’s disease
Simoa	Single Molecule array
MoCA	Montreal Cognitive Assessment
CSF	Incerebrospinal fluid
FTLD	Frontotemporal lobar degeneration
MCI	Mild cognitive impairment
PD	Parkinson's disease
MRI	Magnetic Resonance Imaging
MTA	Medial temporal atrophy
NC	Normal control
CLAS	China Longitudinal Aging Study
NIA-AA	National Institute on Aging-Alzheimer’s Association
CDR	Clinical Dementia Rating
LEAP	Learning Embedding for Atlas Propagation

8. Declarations

8.1 Ethics approval and consent to participate

This study was conducted in accordance with the principles of Declaration of Helsinki, and approved by the Research Ethical Committee of the affiliated mental health center of Shanghai Jiaotong University School of Medicine. All participants had signed the informed consent written informed consent before the start of the study.

8.2 Consent for publication

Not applicable.

8.3 Availability of data and materials

The data base generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

8.4 Competing interests

The authors declare that they have no competing interests.

8.5 Funding

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8.6 Authors' contributions

Wei Li and Lin Sun contributed to the study concept and design. Ling Yue acquired the data. Ye

Wu collected the data. Shifu analyzed the data and drafted the manuscript. All authors have read and approved the final manuscript.

8.7 Acknowledgement

Not applicable.

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Tables

Table 1. General demographic data and clinical characteristics of subjects

Characteristics	aMCI (n=43)	AD (n=35)	Normal (n=30)	p
Age, years	72.21±6.151	71.80±10.737	70.00±4.202	0.451
Education, years	11.84±3.804	12.14±5.621	12.33±2.631	0.316
Male, n(%)	17(39.5)	8(22.9)	11(36.7)	0.269
Smoker, n(%)	7(16.3)	7(6.5)	4(3.7)	0.769
Drinker,n(%)	10(9.3)	5(4.6)	2(1.9)	0.153
Tea drinker, n(%)	19(44.2)	10(28.6)	15(50)	0.181
Hypertension,n(%)	28(65.1)	9(25.7)	16(53.3)	0.002*
Diabetes, n(%)	5(11.6)	3(8.6)	5(16.7)	0.603
Moca	20.48±3.430	5.71±4.908	25.75±2.382	<0.001*
Left hippocampus,mm^3	3401.71±389.866	2495.03±466.353	3383.87±189.653	<0.001*
Right hippocampus,mm^3	3409.89±388.144	2684.93±418.750	3605.23±201.807	<0.001*
Left Amygdala,mm^3	1275.81±250.030	688.208±248.970	1219.90±103.600	<0.001*
Right Amygdala,mm^3	1345.66±173.892	873.992±199.471	1423.06±217.733	<0.001*
Plasma NFL, pg/ml	15.92±6.521	29.42±23.182	12.85±2.138	<0.001*

Table 2. Multiple comparison among three groups(aMCI, AD and Normal)

Variables	Group 1	Group 2	Mean difference	Standard error	p	95%confidence interval
MoCA	aMCI	AD	14.762	0.912	<0.001*	12.95~16.57
		Normal	-5.274	0.972	<0.001*	-7.20~-3.35
	AD	Normal	-20.036	1.010	<0.001*	-22.04~-18.03
Left hippocampus	aMCI	AD	906.682	163.441	<0.001*	574.53~1238.83
		Normal	17.84	177.932	0.921	-343.76~379.44
	AD	Normal	-888.84	186.086	<0.001*	-1267.01~-510.67
Right hippocampus	aMCI	AD	724.968	151.568	<0.001*	416.95~1032.99
		Normal	-195.34	165.006	0.245	-530.673~139.99
	AD	Normal	-920.308	172.568	<0.001*	-1271.01~-569.61
Left Amygdala	aMCI	AD	587.605	96.963	<0.001*	390.552~784.658
		Normal	55.913	105.560	0.600	-158.611~270.437
	AD	Normal	-531.692	110.397	<0.001*	-756.046~-307.337
Right Amygdala	aMCI	AD	471.668	79.833	<0.001*	309.428~633.909
		Normal	-77.396	86.912	0.379	-254.021~99.230
	AD	Normal	-549.064	90.894	<0.001*	-733.783~-364.345
Plasma NFL	aMCI	AD	-13.495	3.157	<0.001*	-19.755~-7.236
		Normal	3.075	3.299	0.353	-3.465~9.616
	AD	Normal	16.570	3.450	<0.001*	9.729~23.411

Table3. Results of partial correlation analysis (hypertension controlled)

Variable 1	Variable 2	relativity	p
Plasma NfL	Moca	-0.415	0.013*
	Left hippocampus	-0.207	0.232
	Right hippocampus	-0.355	0.036*
	Left Amygdala	-0.207	0.232
	Right Amygdala	-0.337	0.048*

Figures

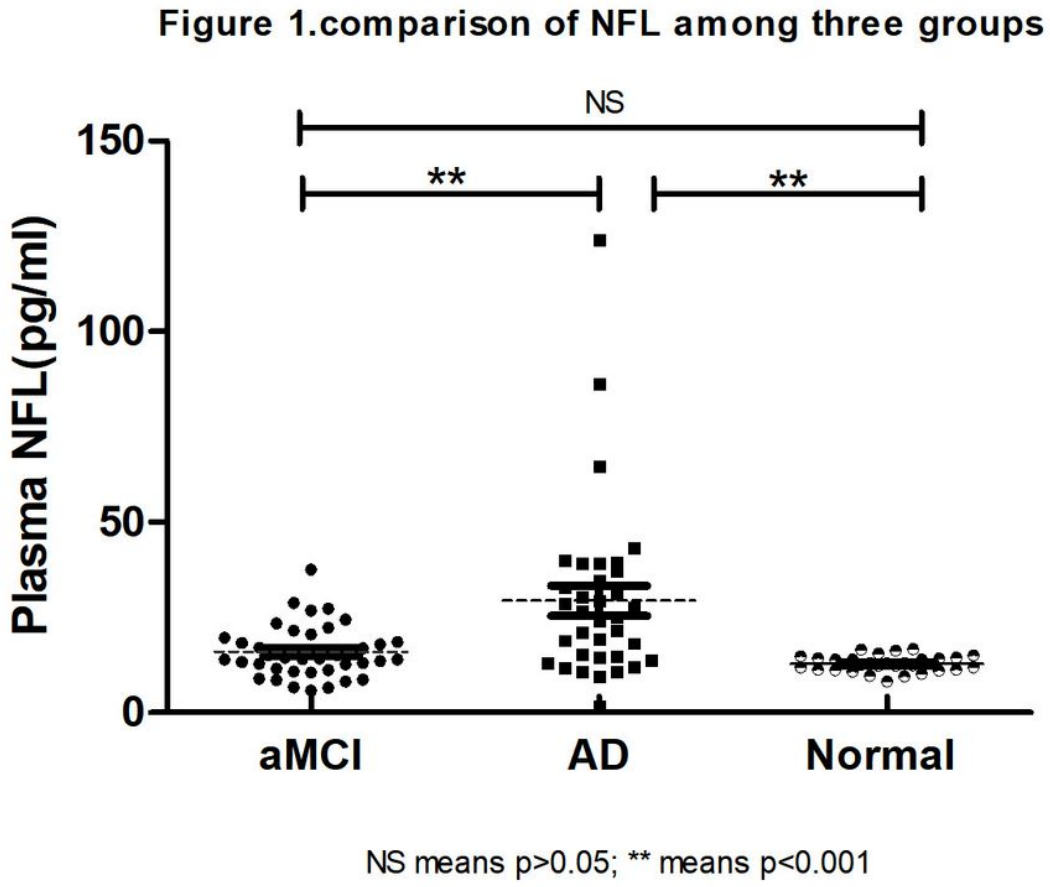

Figure 1

Figure 2. Correlation of NfL with hippocampus and amygdala

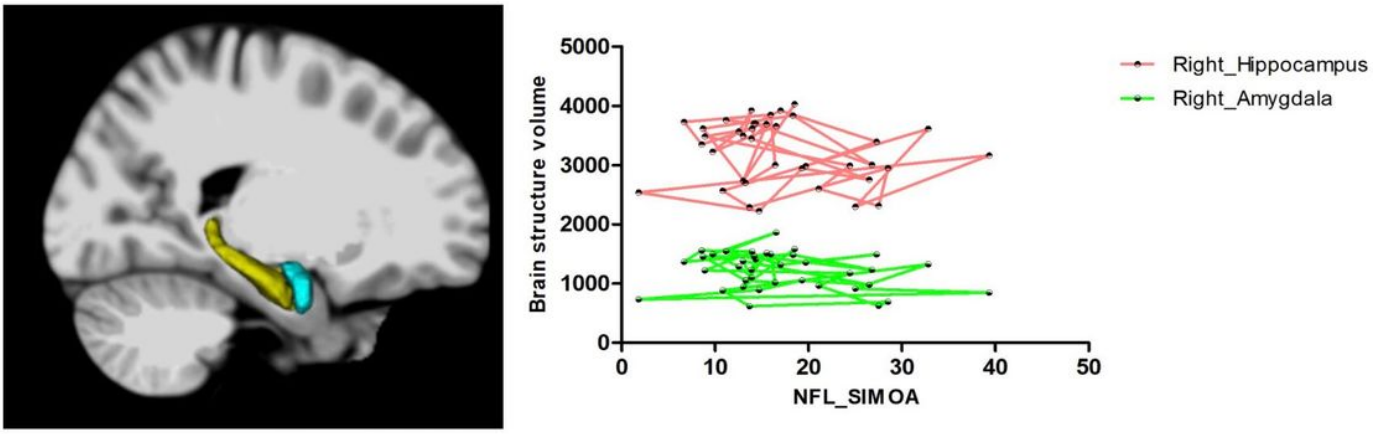

Figure 2