Original Article
Correlation of the expression of NLRP3 mNRA inflammasome and its downstream inflammatory factors in peripheral blood with cognition and activity of daily living of patients with Alzheimer's disease

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Abstract: Objective: To explore the correlation of the expression of nod-like roll receptor3 (NLRP3) and its downstream factors in peripheral blood with cognition and activity of daily living of patients with Alzheimer's disease (AD). Methods: A total of 76 AD patients were enrolled as an AD group, and 76 healthy individuals were enrolled as a control group. The two groups were compared in the levels of peripheral blood NLRP3 and cysteine-requiring aspartate protease-1 (Caspase-1) mRNA, serum interleukin-1β (IL-1β), interleukin-18 (IL-18), and scores of the mini-mental state examination (MMSE), the Montreal cognitive assessment (MoCA), and the activities of daily living (ADL) scales. In addition, the correlation of NLRP3, Caspase-1, IL-1β, and IL-18 with scores of MMSE, MoCA, and ADL scales in the AD group was analyzed, and multivariate logistic regression analysis was carried out on indexes of the two groups with significant differences in univariate analysis to analyze risk factors for AD. Results: The AD group showed higher levels of NLRP3, Caspase-1, IL-1β, and IL-18, and lower score of each scale, and the levels of NLRP3, Caspase-1, IL-1β, and IL-18 were negatively correlated with the score of each scale. In addition, the increase in the levels of NLRP3, Caspase-1, IL-1β, and IL-18, low education level, having a cerebrovascular disease history, having a family history of AD, social activity frequency equal to 1 time per week or less, amount of exercise lower than 30 min per day, and negative life events were risk factors for AD. Conclusion: NLRP3 inflammasome and its downstream factors are strongly linked to the cognition and activity of daily living of AD patients, and they may be involved in the development and progression of AD.

Keywords: NLRP3 protein, Alzheimer's disease, cognition, ability of daily living

Introduction

Alzheimer's disease (AD) is a common irreversible neurodegenerative disease, which is mainly manifested as cognitive impairment, progressive memory loss, affective disorder, and inattention [1]. According to epidemiological data, there are 47,000,000 AD patients worldwide, and the number of them is expected to reach 130,000,000 in 2050. Due to the large population base and accelerated process of social aging in China, the number of AD patients in China has ranked the first in the world [2-4]. At present, there is no gold standard to diagnose early AD, and the etiology and pathogenesis of this disease are still under investigation. There

is a traditional view that the deposition of extracellular amyloid protein β (Aβ), functional loss of nerve synapse, tangles of fibrin in nerve cells and abnormal death of neuronal cells are the main pathological features leading to AD [5, 6]. However, in the past decades, it has been found that neuroimmunity also plays an important role in the pathological development and progression of AD, which provides a novel insight for the pathogenesis and treatment of AD. Some early studies have revealed that cytokines (interleukin-1β (IL-1β) and interleukin-18 (IL-18)) are closely related to the development and progression of AD. Microglia are important innate immune cells in the brain, with a strong connection with neurons, blood vessels, and

astrocytes, which play an important role in the central nervous system. For example, they play a role in the blood brain barrier to maintain the homeostasis of internal environment in the brain, and inflammation can destroy the blood brain barrier and lead to brain diseases. In 2008, one study by Halle et al. [7] found that the chronic deposition of Aβ can stimulate the activity of microglia, thus activating the inflammatory complex of nucleotide binding oligomerization domain nod-like roll receptor 3 (NLRP3) of microglia, and finally activating the cysteine-requiring aspartate protease-1 (Caspase-1) and mediating the expression of its downstream factors, IL-1β and IL-18. NLRP3 can also reduce the degradation of Aβ and further activate it, thus aggravating AD [8-10]. Based on the above, this study explored the expression of peripheral blood NLRP3 mRNA and related downstream inflammatory factors, as well as the correlation of the expression of NLRP3 mNRA and its downstream inflammatory factors with cognition and activity of daily living of AD patients.

Materials and methods

General data

A total of 76 AD patients admitted to Aerospace Center Hospital from January 2017 to January 2019 were enrolled as an AD group, and 76 individuals with cognitive function matching their age and sex were enrolled as a control group. The AD group consisted of 47 males and 29 females, with an average age of 73.34±5.53 years, while the control group consisted of 45 males and 31 females, with an average age of 73.11±5.94 years. There was no significant difference between the two groups in age and sex ratio (both P>0.05). All the participants and their families signed informed consent forms after understanding this study, and the study was approved by the Ethics Committee of Aerospace Center Hospital.

The inclusion criteria for the AD group: Patients meeting relevant diagnosis criteria recommended by the National Institute of Neurological and Communicative Diseases and Stroke/ Alzheimer's Disease and Related Disorders Association [11], including dementia symptoms and manifestations, occult onset, history of cognitive impairments confirmed through reports and observation, amnesia performance

and non-amnesia performance of the initial and most prominent cognitive impairment according to medical history and examination, and patients diagnosed with AD for the first time.

The exclusion criteria for the AD group: Patients comorbid with other types of dementia or with other types of cognitive impairment, patients with a history of mental disease, patients with liver or kidney diseases, anemia, infectious diseases or hyperthyroidism, patients who had taken addictive or analgesic drugs for a long term, patients suffering from diseases that may cause inflammatory reactions, such as common cold, pneumonia and arthritis, and those who had taken hormones and other drugs that may affect inflammation within half a year.

Data collection

The general data of the two groups of participants were collected, including age, sex, educational level, smoking and drinking history, hypertension history, diabetes mellitus history, cardiovascular disease history, cerebrovascular disease history, a family history of AD, inhabiting information, social activity participation once a week or less within the last year or more than once a week within the year, amount of exercise (less than 30 min each day within the last year or 30 min each day or more within the year), negative life events (unpleasant individual events).

Assessment

All the participants were assessed with the mini-mental state examination (MMSE), Montreal cognitive assessment (MoCA), and activities of daily living (ADL) scales, respectively, under the guidance of doctors trained based on unified normal neuropsychological scale before being enrolled in the study.

The MoCA scale was mainly used to evaluate the cognitive impairment of participants [12], which covered 11 items including memory, language, and executive ability, with a total of 30 points. A lower score indicated severer cognitive impairment, and a patient with a score ≥26 points was considered normal.

The MMSE scale covered many items including memory, recall, orientation, language ability,

Table 1. Primer sequences

Primer	Sequences (5'-3')
NLRP3 forward primer	GCTGGTCTTGAATTCCTCA
NLRP3 reverse primer	GGCAACGGATGAGTCTTT
Caspase1 forward primer	CCAGGACATTAAAATAAGCAAACTGT
Caspase1 reverse primer	CCAAAAACCTTTACAGAAGAATCTC
β-actin forward primer	ACTCTTCCAGCCTTCCTTCC
β-actin reverse primer	CGTACAGGTCTTTGCGGATG

Note: NLRP3: nod-like roll receptor 3; Caspase-1: cysteine-requiring aspartate protease-1.

Table 2. Comparison of MMSE, MoCA and ADL scale scores between the two groups

Groups	MMSE	MoCA	ADL
Control group (n=76)	27.45±0.94	28.73±1.14	77.94±3.38
AD group (n=76)	19.50±2.01	19.21±2.21	49.16±3.52
T	31.234	33.375	51.413
P	<0.001	<0.001	<0.001

Note: AD: Alzheimer's disease; MMSE: mini-mental state examination; MoCA: montreal cognitive assessment; ADL: activities of daily living.

attention, and calculation ability, with a total score of 30 points [13]. A lower score indicated a more serious mental disorder, and a patient g with a score ≥27 points was considered normal.

The ADL scale was mainly used to evaluate participants' ability of daily living, with a score of 0-100 points [14]. A lower score indicated worse self-care ability.

Determination of the concentration of inflammatory factors and mRNA

Two tubes of fasting venous blood (3 mL per tube) was sampled from each participant in the morning on the day before enrollment, and each tube of blood was centrifuged at 3000 r/min for 5 min to separate the serum. The levels of IL-1β and IL-18 in the serum were determined by an enzyme-linked immunosorbent assay with a Spectra-MaxParadigm multifunctional microplate reader (Molecular Devices Company, United States). The kits were all purchased from Beyotime Biotechnology (Shanghai) Co., Ltd. RNA was extracted from peripheral blood in one tube using a RNA extraction kit, and a reverse transcription-polymerase chain reaction (RT-PCR) was carried out to the RNA according to the SYBR RT-PCR Kit instructions under the following reaction systems: Pre-

denaturation at 95°C for 30 s, followed by 35 cycles of 95°C for 5 s and 60°C for 30 s. The relative expression of NLRP3 and Caspase1 in each group was determined using the $2^{-\Delta\Delta Ct}$ method with β-actin as an internal reference. Each sample was determined three times, and the results were averaged. The primer sequences are shown in **Table 1**.

Observation indexes

This study mainly analyzed relevant risk factors for AD, and also analyzed the changes of NLRP3 mRNA expression in peripheral blood in AD patients and the correlation of the expression and inflammatory factors with cognitive function.

Statistical analyses

Statistical analyses were carried out using SPSS22.0. Enumeration data are expressed as the number of cases or percentage, and measurement data are expressed as the mean ± standard deviation ($\overline{x}±$ sd). Enumeration data were analyzed using the independent-samples T test, and compared using the χ^2 test. The indexes with statistical differences in univariate analysis were included as variables and further analyzed by multivariate logistic regression, and Pearson's correlation analysis was carried out to analyze the correlation of NLRP3, Caspase-1, IL-1β, IL-18 with scores of MMSE, MoCA, and ADL scales in the AD group. P<0.05 indicates a significant difference.

Results

Comparison of MMSE, MoCA, and ADL scale scores between the two groups

The results showed that the scores of MMSE, MoCA, and ADL scales in the AD group were significantly lower than those in the control group (all P<0.05, **Table 2**).

Comparison of NLRP3, Caspase-1 mRNA, serum IL-1β, and serum IL-18 between the two groups

The results showed that the levels of NLRP3, Caspase-1 mRNA, serum IL-1β, and serum IL-18 in the AD group were significantly higher than those in the control group (all P<0.05, **Table 3**).

Table 3. Comparison of NLRP3 and Caspase-1 mRNA, serum IL-1β, and IL-18 levels in the two groups

Groups	NLRP3	Caspase-1	IL-1β (ng/L)	IL-18 (ng/L)
Control group (n=76)	1.02±0.28	0.97±0.33	2.71±0.54	4.36±1.08
AD group (n=76)	1.79±0.22	1.99±0.28	12.42±2.38	19.30±3.25
T	18.851	20.547	34.686	38.03
P	<0.001	<0.001	<0.001	<0.001

Note: AD: Alzheimer's disease; NLRP3: nod-like roll receptor 3; Caspase-1: cysteine-requiring aspartate protease-1; IL-1β: serum interleukin-1β; IL-18: interleukin-18.

Table 4. Correlation analysis results

Indexes	MMSE	MoCA	ADL
NLRP3	-0.871	-0.886	-0.423
Caspase-1	-0.504	-0.525	-0.711
IL-1β	-0.815	-0.706	-0.745
IL-18	-0.823	-0.806	-0.793

Note: NLRP3: nod-like roll receptor 3; Caspase-1: cysteine-requiring aspartate protease-1; IL-1β: serum interleukin-1β; IL-18: interleukin-18; MMSE: mini-mental state examination; MoCA: montreal cognitive assessment; ADL: activities of daily living.

Correlation analysis

In the AD group, the levels of NLRP3, Caspase-1 mRNA, serum IL-1β, and serum IL-18 were all negatively correlated with MMSE, MoCA and ADL scale scores (**Table 4** and **Figure 1**).

Comparison of general data between the two groups

The results showed that there were significant differences between the two groups in educational level, drinking history, diabetes mellitus history, cerebrovascular disease history, family history of AD, social activity frequency, amount of exercise, and negative life events (all P<0.05, **Table 5**).

Logistic regression analysis

The indexes with statistical differences in univariate analysis were assigned as follows: In terms of education level, illiteracy, primary school level, secondary school level, and college level and above were assigned with 0, 1, 2, and 3, respectively; in terms of drinking history, having no drinking history and having a drinking history were assigned with 0 and 1, respectively; in terms of diabetes mellitus history, having no diabetes mellitus history and having a diabetes mellitus history were assigned with 0 and 1,

respectively; in terms of cerebrovascular disease history, having no cerebrovascular disease history and having a cerebrovascular disease history were assigned with 0 and 1, respectively; in terms of family history of AD, having no family history of AD and having a family history of AD were assigned with 0 and 1, respectively; in terms of social activity, frequency more than 1 time per week and frequency equal to 1 time per week or less were assigned with 0 and 1, respectively; in terms of amount of exercise, frequency equal to 30 min each day or more and frequency less than 30 min each day were assigned with 0 and 1, respectively; in terms of negative life events: Having no negative life event and having negative life events were assigned with 0 and 1, respectively. The value of the control group plus 1 standard deviation was taken as the cutoff value. The peripheral blood NLRP3 RNA smaller than 1.3 and that equal to 1.3 or more were assigned with 0 and 1, respectively; the peripheral blood Caspase-1 RNA smaller than 1.3 and that equal to 1.3 or more were also assigned with 0 and 1, respectively; serum IL-1β level smaller than 3.25 ng/L and that equal to 3.25 ng/L or more were also assigned with 0 and 1, respectively; serum IL-18 level smaller than 5.44 ng/L and that equal to 5.44 ng/L or more were assigned with 0 and 1, respectively. After assignment, logistic regression analysis was carried out.

The results of multivariate logistic regression analysis showed that peripheral blood NLRP3 RNA equal to 1.3 or more, peripheral blood Caspase-1 RNA equal to 1.3 or more, serum IL-1β level equal to 3.25 ng/L or more, serum IL-18 equal to 5.44 ng/L or more, low education level, having a cerebrovascular disease history, having a family history of AD, less social activities (≤1 time/week), less exercise (<30 min/d), and negative life events were risk factors for the development of AD. See **Table 6**.

Discussion

With the development of society, AD has become a global focus. With a very complex etiology, AD involves many aspects including soci-

Correlation between NLRP3 and cognition and life ability of Alzheimer's disease
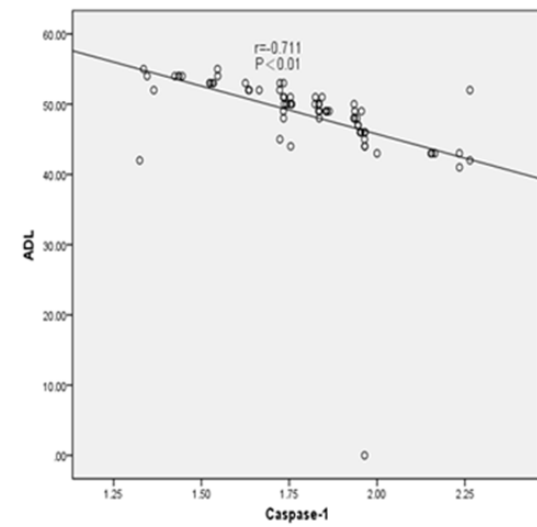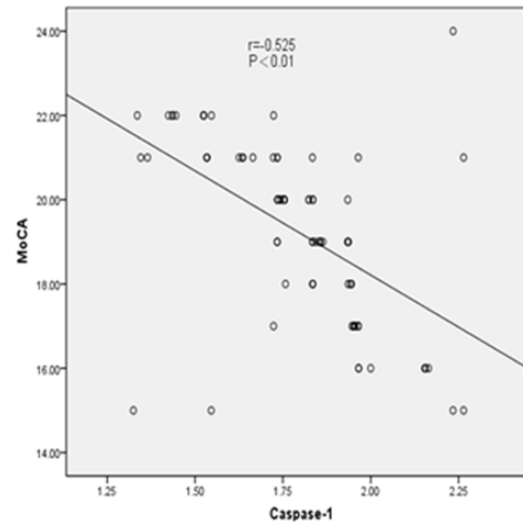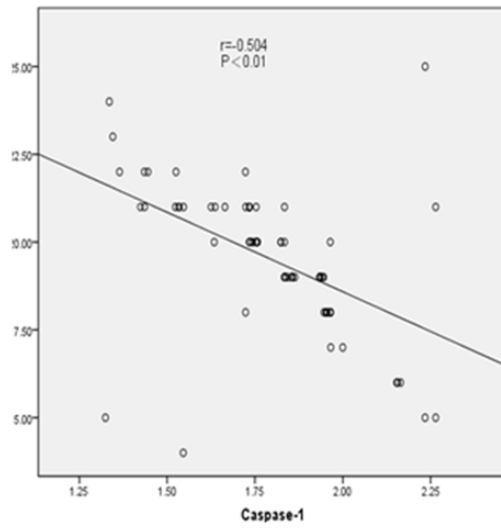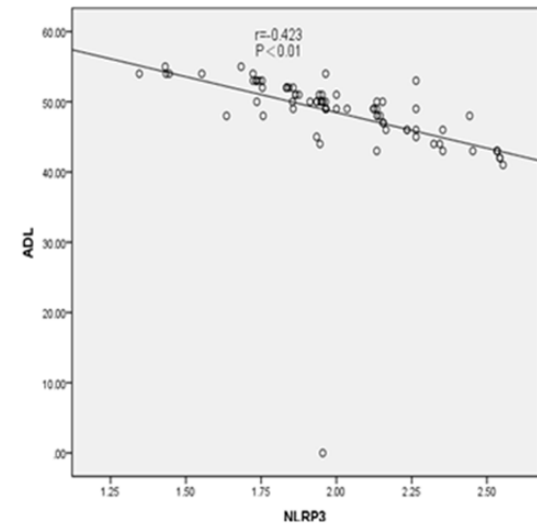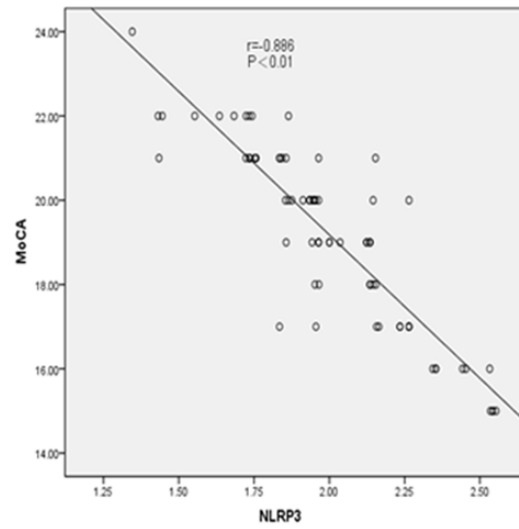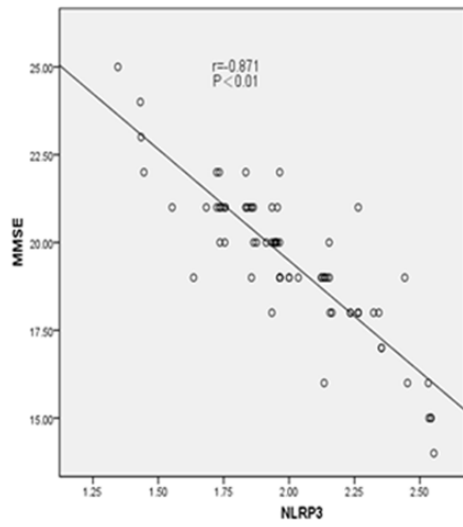
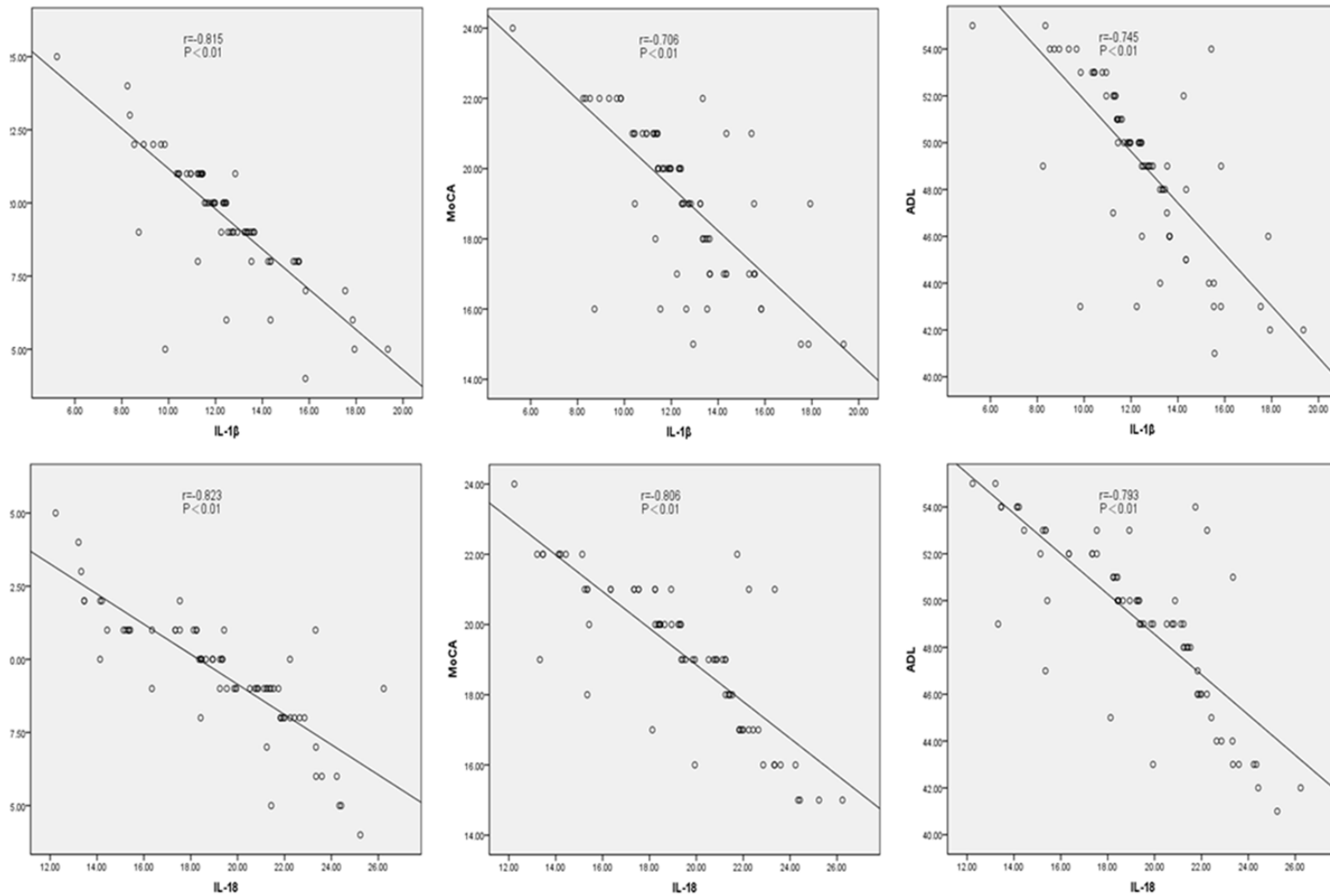# Correlation between NLRP3 and cognition and life ability of Alzheimer's disease

Figure 1. Correlation analysis results.

Table 5. Comparison of two groups of general information

Indexes	AD group (n=76)	Control group (n=76)	Statistics	P
Educational level				
Illiteracy	12	3		
Primary school education	17	13		
High school degree	33	34		
College and above	14	26	9.548	0.023
Smoking history				
Yes	25	16		
No	51	60	2.705	0.100
Drinking history				
Yes	23	12		
No	53	64	4.491	0.034
Diabetes mellitus history				
Yes	28	15		
No	48	61	5.481	0.019
Cardiovascular disease history				
Yes	17	11		
No	59	65	1.576	0.209
Cerebrovascular disease history				
Yes	16	5		
No	60	71	6.686	0.0097
High blood pressure history				
Yes	32	22		
No	46	54	2.466	0.116
A family history of AD				
Yes	22	5		
No	54	71	13.016	0.0003
Solitary				
Yes	19	14		
No	57	62	0.968	0.325
Social activity frequency				
≤1 time/week	34	18		
≥2 time/week	42	58	7.483	0.0062
Amount of exercise				
<30 min/d	37	19		
≥30 min/d	39	57	9.161	0.0025
Negative life events				
Yes	26	11		
No	50	65	8.038	0.0046

Note: AD: Alzheimer's disease.

effectively promote the metabolism of brain cells in the elderly, thus improving their cognitive function, and activities can improve the perception of the brain to external things, thus giving full play to the brain function and reducing the possibility of suffering from AD. In addition, some reports have indicated that with the increase of age, the body's functions decline and the brain tissue degenerates. They have also indicated that the educational level is often closely related to the interest in the surrounding things of the elderly, and their perception and thinking activities, and a high educational level reduces the possibility of suffering from dementia to a certain extent [15, 16]. The results of this study revealed that low education level was a risk factor for AD, which is consistent with the above statements. Cerebrovascular diseases often overlap and interact with the pathogenesis of AD, so patients with one such disease face a higher risk of suffering from AD. Moreover, patients with a family history of AD also face a higher risk of suffering from it than those without such a family history. Some studies have concluded that genetic factors, living environment, and lifestyle all play crucial roles in the development of AD in the family [17-19]. Additionally, this study found that negative life events may also increase the risk of the development of AD, which may be due to the fact that negative life events would aggravate patients' negative emotions, thus indirectly affecting the occur-

ology and biology. Previous studies have explored its risk factors, but the research conclusion is still controversial. The results of this study revealed that less exercise and less social activities were the risk factors affecting the development of AD. Some studies have found that exercise and social activities can

Table 6. Logistic regression analysis results

Indexes	β	SE	Wald	P	OR	95% CI
Educational level	1.157	0.542	16.432	<0.001	3.147	2.434, 7.845
Cerebrovascular disease history	0.813	0.554	12.328	<0.001	3.782	1.983, 6.433
A family history of AD	1.984	0.973	22.849	<0.001	5.786	3.345, 11.874
Social activity frequency (≤1 time/week)	0.823	0.394	5.231	0.002	2.611	1.098, 5.832
Amount of exercise (<30 min/d)	1.298	0.657	13.243	<0.001	2.845	1.387, 6.549
Negative life events	1.014	0.482	7.884	0.001	3.076	2.056, 7.842
NLRP3 mRNA (≥1.3)	1.124	0.221	18.343	<0.001	3.563	1.229, 5.843
Caspase-1 mRNA (≥1.3)	1.198	0.394	16.754	<0.001	3.984	1.092, 5.512
IL-1β (≥3.25 ng/L)	1.443	0.419	17.742	<0.001	2.853	1.225, 4.573
IL-18 (≥5.44 ng/L)	0.985	0.684	14.453	<0.001	3.187	1.396, 6.432

Note: AD: Alzheimer's disease; NLRP3: nod-like roll receptor 3; Caspase-1: cysteine-requiring aspartate protease-1; IL-1β: serum interleukin-1β; IL-18: interleukin-18; SE: state examination; OR: odd ratio.

rence of AD, but this factor still needs further confirmation in subsequent studies.

Microglia are considered to be the main cell type that plays an important role in the regulation of immune inflammation in brain tissues, which secrete various cytokines to promote their action on the neuronal membrane or on receptors in the membrane, thus participating in the function of neurons and affecting the survival of them. Cytokines generated in the process of neuronal apoptosis or necrosis can act on microglia to form a mutual regulation mechanism of neuron-glial network, thus maintaining the homeostasis of the internal environment in the brain. The concept of "inflammasome" was first proposed by Tschopp et al. It is mainly used to describe the activation of immune cells or a class of macromolecular complexes in their cytoplasm that mediate Caspase-1 activation. In recent years, the development and progression of inflammasomes in inflammation-related diseases has become a hot topic in clinical research. NLRP3 inflammasome mainly consists of NLRP3, linker protein (apoptotic speck protein (ASC)) and cysteine-requiring aspartate protease-1 (Caspase-1), which is mainly expressed by microglia in the central nervous system. One study has pointed out that NLRP3 may be closely related to the promotion of microglia on Aβ [20], and one animal experiment has uncovered that NLRP3 inhibitor can inhibit the deposition of Aβ protein in APP/PS1 transgenic mice and significantly improve the cognitive function of the mice, and it can also inhibit the activity of microglia and inflammasomes in the mice to some extent [21]. There is also a study finding that the

expression of Caspase-1 in mice with NLRP3 knocked out decreases, and the deposition of Aβ protein also decreases [22]. A previous study has already explored the expression of NLRP3 in the brain [20]. Differently, this study took peripheral blood as the test sample, with the goal of finding more convenient indicators for clinical diagnosis and treatment of AD. The results showed that compared with normal individuals, AD patients showed higher levels of NLRP3 and Caspase-1 mRNA in peripheral blood monouclear cells, and their levels were negatively correlated with cognitive function and ADL score, which suggested that NLRP3 was closely related to the development and progression of AD.

IL-1β and IL-18 are downstream products of NLRP3, which can promote the proliferation of macrophages [23]. When the central nervous system of the body suffers from injury or inflammatory reaction, microglia and astrocytes collect and phagocytize nerve injury-related factors, thus protecting the central nervous tissues. At the same time, they release a large amount of pronflammatory factors to promote the expression of macrophages and participate in this process, which is the primary stage of neuroinflammation and is mainly mediated by inflammasomes. Some studies have found that IL-18 is closely related to the development and progression of AD, and it can induce human neuroblastoma cells to promote the expression of Aβ. Moreover, some studies have reported that IL-1β is also involved in the development and progression of AD, and animal experiments have revealed that the expression of IL-1β significantly increases in the brain tissues of APP/

SP1 transgenic mice [24]. Furthermore, cell experiments have revealed that oligomeric Aβ could promote the production of IL-1β by primary microglia [25]. The results of this study showed that compared with normal individuals, AD patients showed higher levels of serum IL-1β and IL-18, and their levels were negatively correlated with cognitive function and ADL score, which also implied that NLRP3 was closely related to the development and progression of AD.

To sum up, the increase in the levels of NLRP3, Caspase-1, IL-1β, and IL-18, low education level, having a cerebrovascular disease history, having a family history of AD, less social activity (≤1 time/week), less amount of exercise (<30 min/d), and negative life events are risk factors for AD. AD patients show significantly increased levels of NLRP3 inflammasome and downstream factors (Caspase-1, IL-1β, and IL-18), and they are strongly linked to the cognition and activity of daily living of AD patients, suggesting that they may be involved in the development and progression of AD.

However, there are still some deficiencies in this study. For example, the changes of NLRP3, Caspase-1, IL-1β, and IL-18 levels have not been dynamically analyzed, and the differences in the index levels of AD patients before and after treatment are insufficient. Therefore, they still need to be confirmed by subsequent studies.

Disclosure of conflict of interest

None.

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