Tohoku J. Exp. Med., 2025, **266**, 153-160

Histone Deacetylase 4 as a Potential Biomarker for Post-Stroke Cognitive Impairment

Xinfei Duan,[1] Zhongbo Zhang,[1] Jundong Jia,[1] Jingjing Jiang,[2] Jianfei Li,[3] Ke Hu[1] and Runting Niu[4]

[1]Department of Neurology, Handan Central Hospital, Handan, China
[2]Department of Geriatrics, Handan Central Hospital, Handan, China
[3]Department of Radiology, Handan Central Hospital, Handan, China
[4]Department of Dermatology, Handan Central Hospital, Handan, China

Histon deacetylase 4 (HDAC4) modulates memory and cognitive impairment, but its association with post-stroke cognitive impairment (PSCI) is unclear. This study aimed to investigate the potential of HDAC4 for predicting PSCI risk. Sixty-nine PSCI patients and 70 control post-stroke (CPS) patients were enrolled in this case-control study. In all stroke patients, HDAC4 in peripheral blood mononuclear cells was detected by quantitative polymerase chain reaction; T-helper 17 (Th17) cells were detected by flow cytometry, and interleukin-17A was detected by enzyme-linked immunosorbent assay. HDAC4 was reduced in PSCI patients compared with CPS patients (P = 0.001). In total stroke patients, HDAC4 showed negative linkages with age (P = 0.003), history of diabetes (P = 0.012), stroke recurrence (P = 0.001), Th17 cells (P = 0.027), and interleukin-17A (P = 0.002). Additionally, multivariate logistic regression analysis revealed that HDAC4 (per unit) [odds ratio (OR) = 0.438, P = 0.024] was independently associated with a lower PSCI risk, but age (per unit) (OR = 1.061, P = 0.016) and multifocal disease (yes vs. no) (OR = 2.490, P = 0.014) were independently associated with a higher PSCI risk. By receiver operator characteristic curves, HDAC4 had an acceptable value for predicting PSCI risk [area under the curve (AUC) = 0.656, 95% confidence interval = 0.566-0.746]. The combination of HDAC4, age, and multifocal disease showed a good value for predicting PSCI risk (AUC = 0.728, 95% confidence interval = 0.644-0.811). HDAC4 may serve as a potential biomarker for predicting PSCI risk, which could facilitate the early screening and prevention of PSCI, thus promoting the management of stroke.

Keywords: histone deacetylase; post-stroke cognitive impairment; receiver operator characteristic curves; risk prediction; T-helper 17
Tohoku J. Exp. Med., 2025 June, **266** (2), 153-160.
doi: 10.1620/tjem.2024.J120

Introduction

Stroke is a major cause of disability and mortality in human beings. This disease attacks over 12 million cases and induces over 6.5 million deaths annually worldwide (GBD 2019 Stroke Collaborators 2021). The treatments for stroke mainly include thrombolysis or thrombectomy within a certain period of time window for ischemic stroke, as well as blood pressure and blood glucose management plus surgery (if necessary) for hemorrhage stroke (Mendelson and Prabhakaran 2021; Magid-Bernstein et al. 2022). However, patients with stroke may still develop cognitive impairment or even dementia under appropriate treatment (Rost et al. 2022). The occurrence of post-stroke cognitive impairment (PSCI) is associated with neuron cell death, change of synaptic plasticity, inflammatory response, oxidative stress, and dysregulated immune response including high level and infiltration of T-helper 17 (Th17) cells (Mijajlovic et al. 2017; Gottfried et al. 2021; Chi et al. 2023; Goncharov et al. 2024). PSCI may induce sleep disorder, depression, and even physical disability, which also poses a huge burden for caregivers (Lanctot et al. 2020; Stolwyk et al. 2024). Therefore, early prediction of the risk of PSCI is vital for its prevention and intervention.

Received June 12, 2024; revised and accepted October 24, 2024; J-STAGE Advance online publication November 7, 2024
Correspondence: Runting Niu, Department of Dermatology, Handan Central Hospital, No.15, South Zhonghua Avenue, Handan, Hebei 056000, China.
e-mail: nuo56275145@163.com

Histone deacetylase 4 (HDAC4) modulates the transcription of genes through regulating histone acetylation. It participates in the pathogenesis of various diseases, including cancers, diabetes mellitus, and cardiovascular diseases (Gong et al. 2019; Jiang et al. 2020; Cuttini et al. 2023). Moreover, it is also reported that HDAC4 could modulate memory and cognitive impairment (Fitzsimons et al. 2013). For instance, a previous study reported that either overexpression or knockdown of HDAC4 in the mushroom body, a vital structure for memory formation, impaired long-term memory in drosophila. This study suggested that a normal level of HDAC4 was required for the maintenance of long-term memory (Fitzsimons et al. 2013). Another study showed that a high level of HDAC4 in the hippocampus induced neuronal apoptosis, thus facilitating cognitive impairment (Xu et al. 2023). Apart from that, HDAC4 could also regulate cognitive impairment through potentially inhibiting Th17 differentiation. Several studies have revealed that HDAC4 was negatively correlated with Th17 proportion and response (Lu et al. 2015; Pei et al. 2018; Dou et al. 2022). Meanwhile, Th17 cells are responsible for neuroinflammation and infiltration of neutrophiles in the brain to cause cognitive impairment (Cipollini et al. 2019). Based on the above evidence, we supposed that HDAC4 might serve as a biomarker for PSCI.

The peripheral blood mononuclear cells (PBMCs), containing lymphocytes and monocytes, have been considered as a valuable and easy-acquired specimen of biomarkers (Alexovic et al. 2024). Various studies have used PMBCs as specimen to evaluate biomarkers in different diseases such as diabetes, stroke, and cancers (Mosallaei et al. 2022). Considering that HDAC4 might regulate cognitive function through Th17, we assume that HDAC4 in PMBCs might act as a potential biomarker for PSCI.

The current study detected HDAC4 in PSCI and control post-stroke (CPS) patients, aiming to investigate the potential of HDAC4 for predicting the risk of PSCI.

Materials and Methods

Patients

In this case-control study, a total of 69 PSCI patients were enrolled as the case group, which were identified according to a Chinese expert consensus (Chinese Society of Vascular Cognitive Impairment 2021). The inclusion criteria were: 1) diagnosed with stroke through clinical symptoms and imaging examinations; 2) identified as PSCI; 3) aged more than 18 years old; 4) cooperated with blood sample collection. A total of 70 CPS patients were enrolled as the control group. The inclusion criteria of the control group were the same as for the cases except the presence of PSCI. The exclusion criteria for both case and control groups were similar, which included: 1) patients with cognitive impairment before stroke; 2) patients with cancers or hematological malignancies; 3) female patients who were pregnant or lactational. Patients in both groups were enrolled from April 2022 to May 2023 with a ratio set as 1:1. Age and sex were not matched by intervention between PSCI and CPS groups. This study obtained Ethics Committee approval and written informed consent from patients.

Data and sample collection

Clinical characteristics containing demographics and factors that commonly affected cognitive impairment in stroke patients (both groups) were collected. These characteristics included age, sex, body mass index (BMI), nationality, education degree, history of smoke, history of hypertension, history of hyperlipidemia, history of diabetes, multifocal, stroke recurrence, and stroke type. Besides, peripheral blood samples were collected after enrollment, and each sample was divided into two parts for further detection. One part of the blood sample was used to separate peripheral blood mononuclear cells (PBMCs) for detecting HDAC4 expression by quantitative polymerase chain reaction assay and Th17 cells by flow cytometry assay; while the other part was used to separate serum for detecting interleukin-17A (IL-17A) by enzyme-linked immunosorbent assay.

HDAC4 expression detection

The HDAC4 expression in PBMCs was detected using the following kits: Trizol (No. Cat: R0016, Beyotime, China), cDNA Synthesis Kit with gDNA EZeraser (No. Cat: D7180S, Beyotime, China), and Talent qPCR PreMix (SYBR Green) (No. Cat: FP209, TianGen Biotech, China). GAPDH was set as a reference for normalization, and the PBMC HDAC4 expression was figured out via the $2^{-\Delta\Delta Ct}$ method (the median value of CPS was set as control '1'). The list of primers used can be found in Supplementary Table S1.

Statistical analysis

Data analyses were completed using SPSS 26.0 (IBM, USA) software. Data were described as mean ± standard deviation (SD), median with interquartile range (IQR), or number with percentage. The comparison between groups was analyzed by the student t-test, Chi-square test, or Mann-Whitney U test. The correlation analyses were conducted using Spearman's test. All the clinical characteristics of stroke patients were included in the univariate logistic regression analyses. The independent factors of PSCI risk were explored by the forward-stepwise multivariate logistic regression analysis. The predictive ability of HDAC4 expression or the combined factors (independent risk factors in the multivariate analysis) was shown by receiver operator characteristic (ROC) curves. A P value less than 0.05 was considered statistical significance.

Results

Characteristics of patients

PSCI patients had a mean age of 68.5 ± 7.5 years, consisted with 35 (50.7%) males and 34 (49.3%) females; there

were 43 (62.3%) patients with multifocal disease and 21 (30.4%) patients with stroke recurrence. CPS patients had a mean age of 63.9 ± 8.4 years with 45 (64.3%) males and 25 (35.7%) females; 26 (37.1%) patients had multifocal disease and 11 (15.7%) patients had stroke recurrence.

PSCI patients had a higher mean age (P = 0.001), less advanced education degree (P = 0.034), and higher proportions of history of diabetes (P = 0.026), multifocal disease (P = 0.003), and stroke recurrence (P = 0.039) compared with CPS patients. Moreover, the proportion of Th17 cells

Table 1. Clinical characteristics of stroke patients.

Characteristics	Total stroke patients (N = 139)	PSCI patients (n = 69)	CPS patients (n = 70)	Effect size	*P* value
Age (years)	66.2 ± 8.3	68.5 ± 7.5	63.9 ± 8.4	3.428	0.001[a]
Sex				2.616	0.106[b]
Male	80 (57.6)	35 (50.7)	45 (64.3)		
Female	59 (42.4)	34 (49.3)	25 (35.7)		
BMI (kg/m^2)	24.3 ± 3.2	24.7 ± 3.4	24.0 ± 3.1	1.275	0.204[a]
Nationality				0.936	0.333[b]
Minorities	11 (7.9)	7 (10.1)	4 (5.7)		
Han	128 (92.1)	62 (89.9)	66 (94.3)		
Education degree				4.515	0.034[b]
High school and above	67 (48.2)	27 (39.1)	40 (57.1)		
Middle school and below	72 (51.8)	42 (60.9)	30 (42.9)		
History of smoke				2.684	0.101[b]
No	86 (61.9)	38 (55.1)	48 (68.6)		
Yes	53 (38.1)	31 (44.9)	22 (31.4)		
History of hypertension				3.557	0.059[b]
No	36 (25.9)	13 (18.8)	23 (32.9)		
Yes	103 (74.1)	56 (81.2)	47 (67.1)		
History of hyperlipidemia				2.096	0.148[b]
No	81 (58.3)	36 (52.2)	45 (64.3)		
Yes	58 (41.7)	33 (47.8)	25 (35.7)		
History of diabetes				4.941	0.026[b]
No	93 (66.9)	40 (58.0)	53 (75.7)		
Yes	46 (33.1)	29 (42.0)	17 (24.3)		
Multifocal				8.810	0.003[b]
No	70 (50.4)	26 (37.7)	44 (62.9)		
Yes	69 (49.6)	43 (62.3)	26 (37.1)		
Stroke recurrence				4.249	0.039[b]
No	107 (77.0)	48 (69.6)	59 (84.3)		
Yes	32 (23.0)	21 (30.4)	11 (15.7)		
Stroke type				2.048	0.152[b]
Ischemic	117 (84.2)	55 (79.7)	62 (88.6)		
Hemorrhagic	22 (15.8)	14 (20.3)	8 (11.4)		
Th17 cells (%)	2.7 (2.1-4.0)	3.0 (2.4-4.2)	2.4 (2.0-3.6)	2.442	0.015[c]
IL-17A (pg/mL)	63.8 (46.6-102.5)	71.7 (56.5-94.2)	54.9 (42.5-95.1)	2.837	0.005[c]

§1. To test the normality of continuous variables, the Kolmogorov-Smirnov test was employed. Age and BMI were described as mean ± standard deviation, which followed a normal distribution. Th17 cells and IL-17A were described as median (interquartile range), following nonnormal distribution. Other dichotomous variables left in the table were described as numbers (percentages).
§2. The superscript 'a' represented that the student t-test determined the *P* value between PSCI patients and CPS patients, and the corresponding effect size was '*t*'. The superscript 'b' represented that the *Chi-square* test determined the *P* value between PSCI patients and CPS patients, and the corresponding effect size was '*Chi-square*'. The superscript 'c' represented that the *P* value was determined by the Mann-Whitney U test between PSCI patients and CPS patients, and the corresponding effect size was '*Z*'.
§3. The data for Th17 cells were partially missing. In PSCI patients, data for 6 patients were missing, whereas in CPS patients, data for 12 patients were missing.
PSCI, post-stroke cognitive impairment; CPS, control post-stroke; BMI, body mass index; Th17 cells, T helper 17 cells; IL-17A, interleukin-17A.

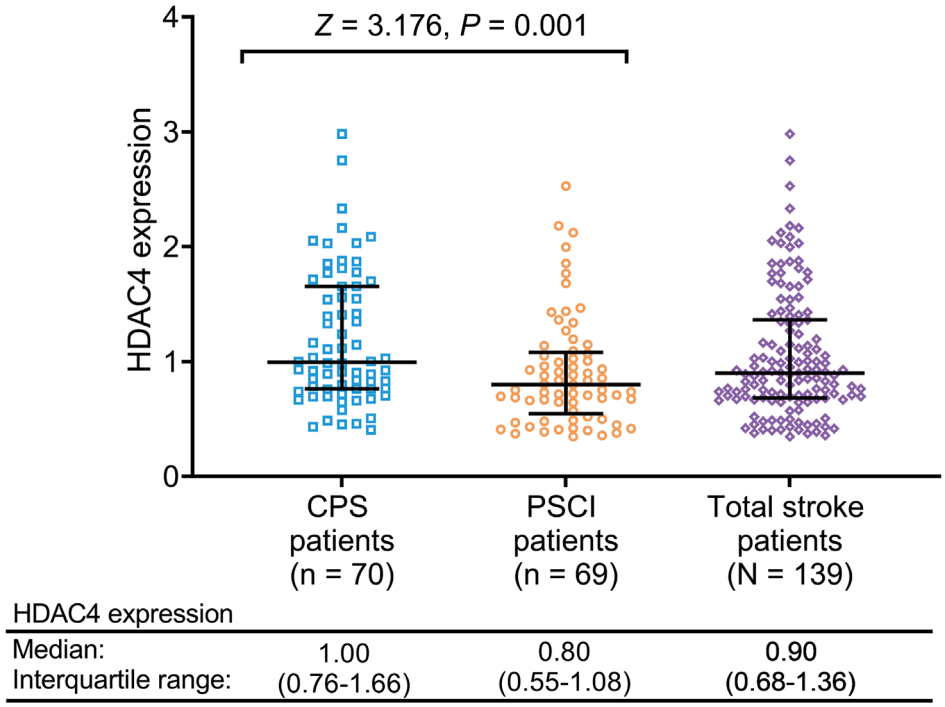

Fig. 1. HDAC4 expression in total stroke patients, PSCI patients, and CPS patients.
Mann-Whitney U test was used to analyze the difference in HDAC4 expression between CPS and PSCI patients.

[median (IQR): 3.0 (2.4-4.2) % vs. 2.4 (2.0-3.6) %, P = 0.015] and IL-17A level [median (IQR): 71.7 (56.5-94.2) pg/mL vs. 54.9 (42.5-95.1) pg/mL, P = 0.005] were both higher in PSCI patients compared with CPS patients. Other characteristics, including sex, BMI, nationality, history of smoke, history of hypertension, history of hyperlipidemia, and stroke type, were not different between PCSI patients and CPS patients. More detailed information is presented in Table 1.

HDAC4 expression in stroke patients

The median (IQR) HDAC4 expression in stroke patients was 0.90 (0.68-1.36). Comparison analysis showed that HDAC4 expression was reduced in PSCI patients compared with CPS patients [median (IQR): 0.80 (0.55-1.08) vs. 1.00 (0.76-1.66), P = 0.001] (Fig. 1).

Relationship of HDAC4 expression with stroke patients' characteristics and Th17 cells

HDAC4 was negatively correlated with age in stroke patients (r = 0.247, P = 0.003). Meanwhile, lower HDAC4 expression was associated with history of diabetes (P = 0.012) and stroke recurrence (P = 0.001). However, no relationship was found in HDAC4 expression with BMI, sex, nationality, education degree, history of smoke, history of hypertension, history of hyperlipidemia, multifocal disease, or stroke type (all P > 0.05) (Table 2).

Moreover, HDAC4 expression was negatively correlated with Th17 cells (P = 0.027) and IL-17A (P = 0.002). Th17 cells and IL-17A were positively correlated with each other (P < 0.001) (Table 3).

Factors affecting PSCI risk

HDAC4 expression (per unit), which means every increase in HDAC4 expression by 1, was associated with a lower PSCI risk [odds ratio (OR) = 0.355, P = 0.003]. Age (per unit) (OR = 1.076, P = 0.001), education degree (middle school and below vs. high school and above) (OR = 2.074, P = 0.035), history of diabetes (yes vs. no) (OR = 2.260, P = 0.028), multifocal disease (yes vs. no) (OR = 2.799, P = 0.003), stroke recurrence (yes vs. no) (OR = 2.347, P = 0.042), and IL-17A (per unit) (OR = 1.013, P = 0.022) were associated with a higher PSCI risk (Table 4). By multivariate logistic regression analysis, HDAC4 expression (per unit) (OR = 0.438, P = 0.024) was independently associated with a lower PSCI risk; while age (per unit) (OR = 1.061, P = 0.016) and multifocal disease (yes vs. no) (OR = 2.490, P = 0.014) were independently associated with a higher PSCI risk (Table 5).

Prediction for PSCI risk

ROC analysis showed that the area under the curve (AUC) [95% confidence interval (CI)] of HDAC4 expression for predicting PSCI risk was of 0.656 (0.566-0.746), indicating an acceptable predictive value (Fig. 2A). Nevertheless, after combining HDAC4 expression with age and multifocal disease, the AUC (95% CI) for predicting PSCI risk increased to 0.728 (0.644-0.811), indicating a good predictive value (Fig. 2B).

Table 2. Correlation of HDAC4 expression with characteristics of stroke patients.

Characteristics	HDAC4 expression	Effect size	*P* value
Continuous variables			
Age	(−)	−0.247	0.003[a]
BMI	(−)	−0.055	0.516[a]
Categorical variables			
Sex		−1.170	0.242[b]
Male	0.89 (0.71-1.52)		
Female	0.90 (0.51-1.20)		
Nationality		−0.343	0.731[b]
Minorities	0.73 (0.68-1.35)		
Han	0.91 (0.68-1.39)		
Education degree		−1.458	0.145[b]
High school and above	0.92 (0.71-1.55)		
Middle school and below	0.87 (0.66-1.19)		
History of smoke		−1.271	0.204[b]
No	0.93 (0.70-1.37)		
Yes	0.79 (0.66-1.32)		
History of hypertension		−1.856	0.063[b]
No	1.05 (0.79-1.74)		
Yes	0.88 (0.67-1.15)		
History of hyperlipidemia		−1.079	0.281[b]
No	0.93 (0.70-1.44)		
Yes	0.84 (0.67-1.21)		
History of diabetes		−2.507	0.012[b]
No	0.93 (0.73-1.42)		
Yes	0.76 (0.46-1.25)		
Multifocal		−1.498	0.134[b]
No	0.92 (0.72-1.38)		
Yes	0.84 (0.65-1.37)		
Stroke recurrence		−3.475	0.001[b]
No	0.95 (0.72-1.47)		
Yes	0.71 (0.46-0.93)		
Stroke type		−0.675	0.500[b]
Ischemic	0.90 (0.69-1.38)		
Hemorrhagic	0.91 (0.50-1.15)		

§1. To test the normality of HDAC4 expression, the Kolmogorov-Smirnov test was employed. HDAC4 expression was described as median (interquartile range), which followed a nonnormal distribution.

§2. The superscript 'a' represented that the Spearman test determined the *P* value between HDAC4 expression and continuous variables, and the corresponding effect size was '*r*'. The superscript 'b' represented that the *P* value was determined by the Mann-Whitney U test between HDAC4 expression and categorical variables, and the corresponding effect size was '*Z*'.

HDAC4, histone deacetylase 4; BMI, body mass index.

Discussion

Previous studies have identified that HDAC4 was dysregulated in patients with diseases associated with cognitive impairment (Shen et al. 2016; Wang et al. 2023a). For instance, it was discovered that HDAC4 was markedly elevated in the brain of patients with Alzheimer's disease (Shen et al. 2016). Another study observed that HDAC4 was increased in the locus coeruleus and substantia nigra of patients with Braak stage 4 and 5 Parkinson's disease (Wang et al. 2023a). The current study revealed that HDAC4 was reduced in PSCI patients compared with CPS patients, which was different from the findings of previous studies (Shen et al. 2016; Wang et al. 2023a). The possible explanation for this discrepancy could be that: previous studies detected HDAC4 in the brain tissues of patients

Table 3. Correlation of HDAC4 expression with Th17 cells and IL-17A of stroke patients.

Items	Th17 cells		IL-17A	
	Effect size	*P* value	Effect size	*P* value
HDAC4 expression	−0.201	0.027	−0.265	0.002
Th17 cells	(−)	(−)	0.413	< 0.001

§. The Spearman test determined the *P* value, and the corresponding effect size was '*r*'.
HDAC4, histone deacetylase 4; Th17 cells, T helper 17 cells; IL-17A, interleukin-17A.

Table 4. Univariate logistic regression analysis of PSCI risk.

Characteristics	OR (95% CI)	*P* value
HDAC4 expression (per unit)	0.355 (0.178-0.710)	0.003
Age (per unit)	1.076 (1.029-1.125)	0.001
Sex (female vs. male)	1.749 (0.886-3.450)	0.107
BMI (per unit)	1.070 (0.964-1.188)	0.204
Nationality (Han vs. minorities)	0.537 (0.150-1.924)	0.339
Education degree (middle school and below vs. high school and above)	2.074 (1.054-4.080)	0.035
History of smoke (yes vs. no)	1.780 (0.890-3.558)	0.103
History of hypertension (yes vs. no)	2.108 (0.964-4.612)	0.062
History of hyperlipidemia (yes vs. no)	1.650 (0.836-3.256)	0.149
History of diabetes (yes vs. no)	2.260 (1.094-4.671)	0.028
Multifocal (yes vs. no)	2.799 (1.408-5.564)	0.003
Stroke recurrence (yes vs. no)	2.347 (1.030-5.344)	0.042
Stroke type (hemorrhagic vs. ischemic)	1.973 (0.769-5.058)	0.157
Th17 cells (per unit)	1.313 (0.991-1.739)	0.057
IL-17A (per unit)	1.013 (1.002-1.024)	0.022

PSCI, post-stroke cognitive impairment; OR, odds ratio; CI, confidence interval; HDAC4, histone deacetylase 4; BMI, body mass index; Th17 cells, T helper 17 cells; IL-17A, interleukin-17A.

Table 5. Multivariate logistic regression analysis of PSCI risk.

Characteristics	OR (95% CI)	*P* value
HDAC4 expression (per unit)	0.438 (0.214-0.897)	0.024
Age (per unit)	1.061 (1.011-1.113)	0.016
Multifocal (yes vs. no)	2.490 (1.205-5.146)	0.014

§. HDAC4 expression, sex, body mass index (BMI), nationality, education degree, history of smoke, history of hypertension, history of hyperlipidemia, history of diabetes, multifocal, stroke recurrence, stroke type, and IL-17A were included in the analysis.
PSCI, post-stroke cognitive impairment; OR, odds ratio; CI, confidence interval; HDAC4, histone deacetylase 4.

with Alzheimer's disease or Parkinson's disease (Shen et al. 2016; Wang et al. 2023a), but the current study compared HDAC4 in the PBMCs of PSCI and CPS patients. The difference in tissue/cell where HDAC4 was hosted could result in the diverse effect of HDAC4 on cognitive impairment. In detail, HDAC4 overexpression in the brain tissues promoted apoptosis of hippocampal neurons and impairs synaptic plasticity to induce cognitive impairment (Mielcarek et al. 2013; Xu et al. 2023); while HDAC4 insufficiency in the cluster of differentiation (CD)4^+ T cells might facilitate the differentiation of Th17 cells to induce cognitive impairment (Dou et al. 2022; Wang et al. 2023b).

The current study also revealed negative linkages of HDAC4 with Th17 cells, IL-17A, age, history of diabetes, and stroke recurrence in stroke patients. The possible explanations were as follows: (1) HDAC4 negatively regulated the differentiation of Th17 cells (Dou et al. 2022), which resulted in its negative correlation with Th17 cells and IL-17A in stroke patients. (2) HDAC4 was negatively correlated with Th17 cells. Meanwhile, Th17 cells were

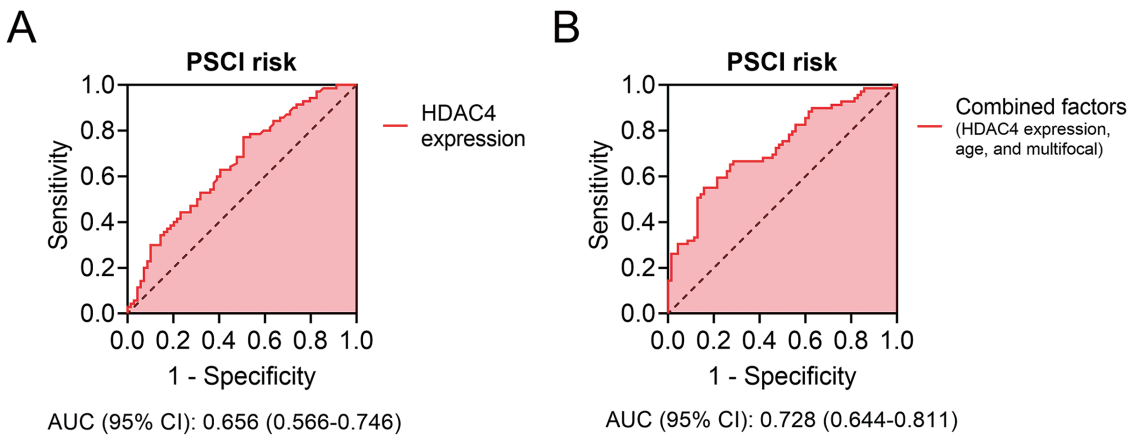

Fig. 2. The ability of HDAC4 to predict PSCI risk.
HDAC4 to predict PSCI risk (A). The combination of HDAC4, age, and multifocal disease to predict PSCI risk (B). ROC analysis with AUCs were used to analyze the predictive value of variables for PSCI risk.

involved in age-associated senescence (Lee et al. 2011; Li et al. 2017). Therefore, our study found that HDAC4 was negatively correlated with age in stroke patients. (3) HDAC4 might inhibit the pathogenesis of diabetes. According to a study, HDAC4 knockdown mice had higher levels of circulating glucose, free fatty acid, and insulin compared with wild-type mice when treated with a high-fat diet. This study suggested that HDAC4 knockdown facilitated insulin resistance and contributed to the pathogenesis of diabetes (Luan et al. 2014). Therefore, our study revealed that HDAC4 was negatively associated with history of diabetes. (4) Th17 cells were associated with stroke recurrence according to a previous study (Yu et al. 2022). Therefore, the current study found that HDAC4 was negatively associated with stroke recurrence.

More importantly, our study also found that HDAC4 showed an acceptable ability to predict the risk of PCSI, and it was independently associated with a lower risk of PCSI. These findings could be explained by the ability of HDAC4 dysregulation to induce cognitive impairment as mentioned above (Mielcarek et al. 2013; Dou et al. 2022; Wang et al. 2023b; Xu et al. 2023). Apart from HDAC4, age and multifocal disease were independently and positively associated with PSCI risk in our study. These variables are well-recognized risk factors for PCSI (Mijajlovic et al. 2017). In order to improve the predictive performance of HDAC4 to the risk of PCSI, we further combined it with age and multifocal disease. The data revealed that performance for predicting PSCI risk was promoted after combining HDAC4 with age and multifocal disease. These findings suggested that the combination of HDAC4, age, and multifocal disease could serve as a predictive tool to identify PSCI risk. In clinical practice, the combination of HDAC4, age, and multifocal could be applied as a predictive model for PSCI risk. Clinicians could use this model to identify patients with a high risk of PSCI, thus implementing more radical treatments to these patients, thus improving their prognosis. Nevertheless, the AUC of HDAC4 combined with age and multifocal disease was still not great enough, which meant that predicting PSCI was not an easy issue. Therefore, further studies are required to identify biomarkers with higher performance to predict PSCI risk.

Our study revealed the potential of HDAC4 for predicting PSCI risk, which had not been reported before. Meanwhile, our study detected HDAC4 from PBMCs, which were easy to acquire. However, several limitations should be clarified. First, the sample size was not large enough, and the predictive value of HDAC4 for PSCI risk should be verified in a larger sample size. Second, the current study did not enroll a validation cohort to confirm the predictive value of HDAC4 for PSCI risk. Third, this study only enrolled stroke patients, and the predictive value of HDAC4 for cognitive impairment in other diseases, such as Alzheimer's disease and Parkinson's disease, should be further investigated. Fourth, the association of HDAC4 in other specimen with PSCI risk, such as cerebrospinal fluid, should be explored in further studies.

Collectively, HDAC4 is negatively linked with Th17 cells, age, history of diabetes, and stroke recurrence in stroke patients; meanwhile, its low expression reflects a high risk of PSCI. The findings of this study indicate that HDAC4 may be applied for the early screening and prevention of PSCI.

Conflict of Interest

The authors declare no conflict of interest.

References

Alexovic, M., Ulicna, C., Sabo, J. & Davalieva, K. (2024) Human peripheral blood mononuclear cells as a valuable source of disease-related biomarkers: Evidence from comparative proteomics studies. *Proteomics Clin. Appl.*, **18**, e2300072.

Chi, X., Wang, L., Liu, H., Zhang, Y. & Shen, W. (2023) Post-stroke cognitive impairment and synaptic plasticity: A review about the mechanisms and Chinese herbal drugs strategies. *Front. Neurosci.*, **17**, 1123817.

Chinese Society of Vascular Cognitive Impairment (2021) Experts Consensus on Post-stroke Cognitive Impairment Management 2021. *Chinese Journal of Stroke*, **16**, 376-389.

Cipollini, V., Anrather, J., Orzi, F. & Iadecola, C. (2019) Th17 and Cognitive Impairment: Possible Mechanisms of Action. *Front. Neuroanat.*, **13**, 95.

Cuttini, E., Goi, C., Pellarin, E., Vida, R. & Brancolini, C. (2023) HDAC4 in cancer: A multitasking platform to drive not only epigenetic modifications. *Front. Mol. Biosci.*, **10**, 1116660.

Dou, B., Ma, F., Jiang, Z. & Zhao, L. (2022) Blood HDAC4 Variation Links With Disease Activity and Response to Tumor Necrosis Factor Inhibitor and Regulates CD4+ T Cell Differentiation in Ankylosing Spondylitis. *Front. Med.* (*Lausanne*), **9**, 875341.

Fitzsimons, H.L., Schwartz, S., Given, F.M. & Scott, M.J. (2013) The histone deacetylase HDAC4 regulates long-term memory in Drosophila. *PLoS One*, **8**, e83903.

Global Burden of Disease (GBD) 2019 Stroke Collaborators (2021) Global, regional, and national burden of stroke and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol.*, **20**, 795-820.

Goncharov, N.V., Popova, P.I., Kudryavtsev, I.V., Golovkin, A.S., Savitskaya, I.V., Avdonin, P.P., Korf, E.A., Voitenko, N.G., Belinskaia, D.A., Serebryakova, M.K., Matveeva, N.V., Gerlakh, N.O., Anikievich, N.E., Gubatenko, M.A., Dobrylko, I.A., et al. (2024) Immunological Profile and Markers of Endothelial Dysfunction in Elderly Patients with Cognitive Impairments. *Int. J. Mol. Sci.*, **25**, 1888.

Gong, M., Yu, Y., Liang, L., Vuralli, D., Froehler, S., Kuehnen, P., Du Bois, P., Zhang, J., Cao, A., Liu, Y., Hussain, K., Fielitz, J., Jia, S., Chen, W. & Raile, K. (2019) HDAC4 mutations cause diabetes and induce beta-cell FoxO1 nuclear exclusion. *Mol. Genet. Genomic Med.*, **7**, e602.

Gottfried, I., Schottlender, N. & Ashery, U. (2021) Hyperbaric Oxygen Treatment-From Mechanisms to Cognitive Improvement. *Biomolecules*, **11**, 1520.

Jiang, H., Jia, D., Zhang, B., Yang, W., Dong, Z., Sun, X., Cui, X., Ma, L., Wu, J., Hu, K., Sun, A. & Ge, J. (2020) Exercise improves cardiac function and glucose metabolism in mice with experimental myocardial infarction through inhibiting HDAC4 and upregulating GLUT1 expression. *Basic Res. Cardiol.*, **115**, 28.

Lanctot, K.L., Lindsay, M.P., Smith, E.E., Sahlas, D.J., Foley, N., Gubitz, G., Austin, M., Ball, K., Bhogal, S., Blake, T., Herrmann, N., Hogan, D., Khan, A., Longman, S., King, A., et al. (2020) Canadian Stroke Best Practice Recommendations: Mood, Cognition and Fatigue following Stroke, 6th edition update 2019. *Int. J. Stroke*, **15**, 668-688.

Lee, J.S., Lee, W.W., Kim, S.H., Kang, Y., Lee, N., Shin, M.S., Kang, S.W. & Kang, I. (2011) Age-associated alteration in naive and memory Th17 cell response in humans. *Clin. Immunol.*, **140**, 84-91.

Li, Q., Ding, S., Wang, Y.M., Xu, X., Shen, Z., Fu, R., Liu, M., Hu, C., Zhang, C., Cao, Q. & Wang, Y. (2017) Age-associated alteration in Th17 cell response is related to endothelial cell senescence and atherosclerotic cerebral infarction. *Am. J. Transl. Res.*, **9**, 5160-5168.

Lu, W., You, R., Yuan, X., Yang, T., Samuel, E.L., Marcano, D.C., Sikkema, W.K., Tour, J.M., Rodriguez, A., Kheradmand, F. & Corry, D.B. (2015) The microRNA miR-22 inhibits the histone deacetylase HDAC4 to promote T(H)17 cell-dependent emphysema. *Nat. Immunol.*, **16**, 1185-1194.

Luan, B., Goodarzi, M.O., Phillips, N.G., Guo, X., Chen, Y.D., Yao, J., Allison, M., Rotter, J.I., Shaw, R. & Montminy, M. (2014) Leptin-mediated increases in catecholamine signaling reduce adipose tissue inflammation via activation of macrophage HDAC4. *Cell Metab.*, **19**, 1058-1065.

Magid-Bernstein, J., Girard, R., Polster, S., Srinath, A., Romanos, S., Awad, I.A. & Sansing, L.H. (2022) Cerebral Hemorrhage: Pathophysiology, Treatment, and Future Directions. *Circ. Res.*, **130**, 1204-1229.

Mendelson, S.J. & Prabhakaran, S. (2021) Diagnosis and Management of Transient Ischemic Attack and Acute Ischemic Stroke: A Review. *JAMA*, **325**, 1088-1098.

Mielcarek, M., Landles, C., Weiss, A., Bradaia, A., Seredenina, T., Inuabasi, L., Osborne, G.F., Wadel, K., Touller, C., Butler, R., Robertson, J., Franklin, S.A., Smith, D.L., Park, L., Marks, P.A., et al. (2013) HDAC4 reduction: a novel therapeutic strategy to target cytoplasmic huntingtin and ameliorate neurodegeneration. *PLoS Biol.*, **11**, e1001717.

Mijajlovic, M.D., Pavlovic, A., Brainin, M., Heiss, W.D., Quinn, T.J., Ihle-Hansen, H.B., Hermann, D.M., Assayag, E.B., Richard, E., Thiel, A., Kliper, E., Shin, Y.I., Kim, Y.H., Choi, S., Jung, S., et al. (2017) Post-stroke dementia - a comprehensive review. *BMC Med.*, **15**, 11.

Mosallaei, M., Ehtesham, N., Rahimirad, S., Saghi, M., Vatandoost, N. & Khosravi, S. (2022) PBMCs: a new source of diagnostic and prognostic biomarkers. *Arch. Physiol. Biochem.*, **128**, 1081-1087.

Pei, X.F., Cao, L.L., Huang, F., Qiao, X., Yu, J., Ye, H., Xi, C.L., Zhou, Q.C., Zhang, G.F. & Gong, Z.L. (2018) Role of miR-22 in intestinal mucosa tissues and peripheral blood CD4+ T cells of inflammatory bowel disease. *Pathol. Res. Pract.*, **214**, 1095-1104.

Rost, N.S., Brodtmann, A., Pase, M.P., van Veluw, S.J., Biffi, A., Duering, M., Hinman, J.D. & Dichgans, M. (2022) Post-Stroke Cognitive Impairment and Dementia. *Circ. Res.*, **130**, 1252-1271.

Shen, X., Chen, J., Li, J., Kofler, J. & Herrup, K. (2016) Neurons in Vulnerable Regions of the Alzheimer's Disease Brain Display Reduced ATM Signaling. *eNeuro*, **3**, ENEURO.0124-15.2016.

Stolwyk, R.J., Mihaljcic, T., Wong, D.K., Hernandez, D.R., Wolff, B. & Rogers, J.M. (2024) Post-stroke Cognition is Associated with Stroke Survivor Quality of Life and Caregiver Outcomes: A Systematic Review and Meta-analysis. *Neuropsychol. Rev.*, **34**, 1235-1264.

Wang, L., Liu, L., Han, C., Jiang, H., Ma, K., Guo, S., Xia, Y., Wan, F., Huang, J., Xiong, N. & Wang, T. (2023a) Histone Deacetylase 4 Inhibition Reduces Rotenone-Induced Alpha-Synuclein Accumulation via Autophagy in SH-SY5Y Cells. *Brain Sci.*, **13**, 670.

Wang, T., Feng, W., Ju, M., Yu, H., Guo, Z., Sun, X., Yang, K., Liu, M. & Xiao, R. (2023b) 27-hydroxycholesterol causes cognitive deficits by disturbing Th17/Treg balance and the related immune responses in mild cognitive impairment patients and C57BL/6J mice. *J. Neuroinflammation*, **20**, 305.

Xu, Y., Wei, S., Zhu, L., Huang, C., Yang, T., Wang, S., Zhang, Y., Duan, Y., Li, X., Wang, Z. & Pan, W. (2023) Low expression of the intestinal metabolite butyric acid and the corresponding memory pattern regulate HDAC4 to promote apoptosis in rat hippocampal neurons. *Ecotoxicol. Environ. Saf.*, **253**, 114660.

Yu, S., Cui, W., Han, J., Chen, J. & Tao, W. (2022) Longitudinal change of Th1, Th2, and Th17 cells and their relationship between cognitive impairment, stroke recurrence, and mortality among acute ischemic stroke patients. *J. Clin. Lab. Anal.*, **36**, e24542.

Supplementary Files

Please find supplementary file(s);
https://doi.org/10.1620/tjem.2024.J120