Received: 14 March 2022 | Revised: 19 April 2022 | Accepted: 21 April 2022

DOI: 10.1002/jcla.24468

RESEARCH ARTICLE

WILEY

Longitudinal variation of circulating lnc-ITSN1-2: A novel biomarker reflecting disease severity, inflammation, recurrence, and death risk in acute ischemic stroke patients

Gang Wang[1] | Ying Zhou[2] | Tingting Zhong[1] | Aixia Song[1] | Qian Xue[1]

[1]Department of Neurology, The First Affiliated Hospital of Hebei North University, Zhangjiakou, China

[2]Department of ICU, The First Affiliated Hospital of Hebei North University, Zhangjiakou, China

Correspondence
Qian Xue, Department of Neurology, The First Affiliated Hospital of Hebei North University, No. 12, Changqing Road, Zhangjiakou 075000, China.
Email: shiqian007783@163.com

Abstract

Background: Long noncoding RNA intersectin 1–2 (lnc-ITSN1-2) regulates inflammation and neuronal apoptosis; meanwhile, the latter two factors participate in the pathogenesis of acute ischemic stroke (AIS). Therefore, this study detected lnc-ITSN1-2 at multiple time points, aiming to explore its longitudinal variation and clinical value in the management of AIS patients.

Methods: The current study enrolled 102 AIS patients, then detected their lnc-ITSN1-2 in peripheral blood mononuclear cell (PBMC) at baseline (D0), day (D)1, D3, D7, month (M)1, M3, M6, and year (Y)1 after admission using RT-qPCR. Additionally, lnc-ITSN1-2 in PBMC of 50 controls was also detected.

Results: Lnc-ITSN1-2 was up-regulated in AIS patients than that in controls ($p < 0.001$). Lnc-ITSN1-2 positively associated with NIHSS score, TNF-α, and IL-17A (all $p < 0.050$) but was not linked with IL-6 ($p = 0.093$) in AIS patients. Notably, lnc-ITSN1-2 was gradually increased from D0 to D3; while it switched to decrease from D3 to Y1 in AIS patients. Lnc-ITSN1-2 disclosed similar longitudinal variation during 1 year in non-recurrent ($p < 0.001$), recurrent ($p = 0.001$), and survived patients ($p < 0.001$), while the variation of lnc-ITSN1-2 in died patients was not obvious ($p = 0.132$). More importantly, lnc-ITSN1-2 at D0, D3, D7, M1, M3, M6, and Y1 was higher in recurrent AIS patients than that in non-recurrent AIS patients (all $p < 0.050$); moreover, lnc-ITSN1-2 at D3, D7, M1, M3, and M6 was up-regulated in died AIS patients than AIS survivors (all $p < 0.050$).

Conclusion: The dynamic variation of lnc-ITSN1-2 could serve as a biomarker reflecting disease severity, inflammatory cytokines, recurrence, and death risk in AIS patients.

KEYWORDS
acute ischemic stroke, dynamic variation, lnc-ITSN1-2, NIHSS score, recurrence and death risk

Gang Wang and Ying Zhou contributed equally to this work.

1 | INTRODUCTION

Stroke, a common cerebrovascular disease, remains the leading cause of death in China, resulting in 150 deaths per 10,000 population in 2017.[1–3] Acute ischemic stroke (AIS), accounting for 75%–90% of all stroke cases, is caused by abnormalities of blood supply to cerebral tissue, whose main etiologies include embolism, large-artery atherosclerosis, and small-artery occlusion.[4,5] At present, the primary treatments for AIS are intravenous thrombolysis, mechanical thrombectomy, antiplatelet treatment, etc., with the goals of revascularization and preventing secondary neuronal injury.[6–8] However, the clinical outcomes of AIS patients are still unsatisfying with a 5-year recurrence rate of approximately 20% and a disability rate ranging from 36% to 71%; meanwhile, recurrence in AIS patients greatly increases the corresponding death rate.[9–12] Hence, exploring novel biomarkers assisting clinicians to monitor disease severity, recurrence, and death risk of AIS patients is meaningful.

Long noncoding RNA intersectin 1–2 (lnc-ITSN1-2), located on human chromosome 21 (451 bp in length), is a newly recognized long noncoding RNA (lncRNA), which is regarded as an inflammation regulator in some inflammatory disorders (such as ankylosing spondylitis (AS) and severe acute pancreatitis (SAP)).[13,14] Besides, one in vivo study finds that the overexpression of lnc-ITSN1-2 promotes inflammation and cell injury in HT22 cells, which implies that lnc-ITSN1-2 may promote neuron apoptosis.[15] Combining that systemic inflammation and neuronal death participate in the pathogenesis of AIS, it is hypothesized that lnc-ITSN1-2 might serve a considerable role in the development of AIS.[16,17] In our preliminary study with a relatively small sample size, we observed an increasing trend of lnc-ITSN1-2 in AIS patients compared with controls. Besides, a recent study observes that increased baseline lnc-ITSN1-2 is related to elevated disease severity and poor survival in AIS patients; while it lacks multiple-time point detection and longitudinal analysis of lnc-ITSN1-2.[18]

Therefore, this study detected lnc-ITSN1-2 at multiple time points, aiming to explore its longitudinal variation and clinical value in the management of AIS patients.

2 | METHODS

2.1 | Subjects

This prospective study consecutively included 102 patients with first episode AIS who were admitted to the hospital between August 2017 and April 2020. Eligible patients were required to meet the following criteria: (1) newly diagnosed as AIS according to the American Stroke Association Guideline[19]; (2) over 18 years old; (3) hospitalized within 24 h after symptoms onset; (4) absent of intracranial hemorrhage; (5) able to provide peripheral blood (PB) samples. Patients who had the following conditions were excluded (1) had inflammatory disease or immune system disease; (2) previously received the immunosuppressive treatment; (3) presented with active infections; (4) concomitant with cancers or hematological malignancies; (5) female patients during lactating or pregnancy. Additionally, a total of 50 subjects with high risk of stroke were recruited in the study as controls. The inclusion criteria for controls were as follows: (a) without diagnosis of stroke; (b) had high risk of stroke, which was defined as having at least two of the following conditions: history of smoke, hypertension, hyperlipidemia, hyperuricemia, diabetes mellitus, chronic kidney disease (CKD), atrial fibrillation, lack of exercise, and family history of stroke; (c) aged more than 18 years. The exclusion criteria for AIS patients were suitable for controls as well. The study was permitted by the Ethics Committee.

2.2 | Collection for data and samples

Clinical characteristics of AIS patients were recorded for study analysis. PB samples of AIS patients were collected after admission (D0), then peripheral blood mononuclear cell (PBMC) and serum were isolated by density gradient centrifugation. Besides, PBMC samples were also collected from AIS patients after 1 day (D1), 3 days (D3), 7 days (D7), 1 month (M1), 3 months (M3), 6 months (M6), and 1 year (Y1) if accessible. In addition, PB samples of controls were obtained after enrollment to separate PBMC samples for subsequent detection.

2.3 | Detection of samples

Peripheral blood mononuclear cell samples of all subjects were applied to determine lnc-ITSN1-2 expression using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted by PureZOL RNA isolation reagent (Bio-Rad), then reserve transcription was completed using iScript™ cDNA Synthesis Kit (with random primer; Bio-Rad). After that, qPCR was achieved by QTaq™ DNA Polymerase Mix (Clontech). The expression of lnc-ITSN1-2 was calculated by the $2^{-\Delta\Delta Ct}$ method, using GAPDH as the internal reference. Besides, qPCR primers were designed referring to the previous study.[20] Additionally, serum samples collected from AIS patients were used to examine the levels of inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and IL-17A. The examinations were performed using enzyme-linked immunosorbent assay (ELISA) with commercial human ELISA Kits (Bio-Techne China Co. Ltd.) according to the instructions provided by manufacturers.

2.4 | Follow-up

All AIS patients were closely followed up for 1 year according to the AIS Guideline,[19] then the continuous follow-up was managed until April 2021. During the follow-up, disease recurrence and patient's death were recorded, then accumulating recurrence rate and accumulating mortality were calculated.

2.5 | Statistics

SPSS (version 24.0, IBM Corp.) was employed for statistical analysis. GraphPad Prism (version 6.01, GraphPad Software Inc.) was employed for graph plotting. Student's *t* test, Mann–Whitney U test and chi-square test were used for comparison analysis; Spearman's rank correlation test or Mann–Whitney U test was used for correlation analysis. Receiver operating characteristic (ROC) curve was constructed to assess lnc-ITSN1-2 expression performance in differentiating participants. Friedman's test was used to assess lnc-ITSN1-2 expression change from D0. Kaplan–Meier method was applied to exhibit accumulating recurrence rate and accumulating mortality. Univariate logistic regression analyses were conducted to investigate the correlation of ITSN1-2 with the recurrence and mortality risk in AIS patients. p value <0.05 was considered significant.

3 | RESULTS

3.1 | Study flow

A total of 118 AIS patients were invited to this study, then 16 patients were excluded; 11 of them were excluded for inclusion criteria or exclusion criteria, and the other 5 patients declined to participate (Figure 1). The remaining 102 patients were included in the study. PBMCs were collected at D0, D1, D3, D7, M1, M3, M6, and Y1 to determine lnc-ITSN1-2 using RT-qPCR; additionally,

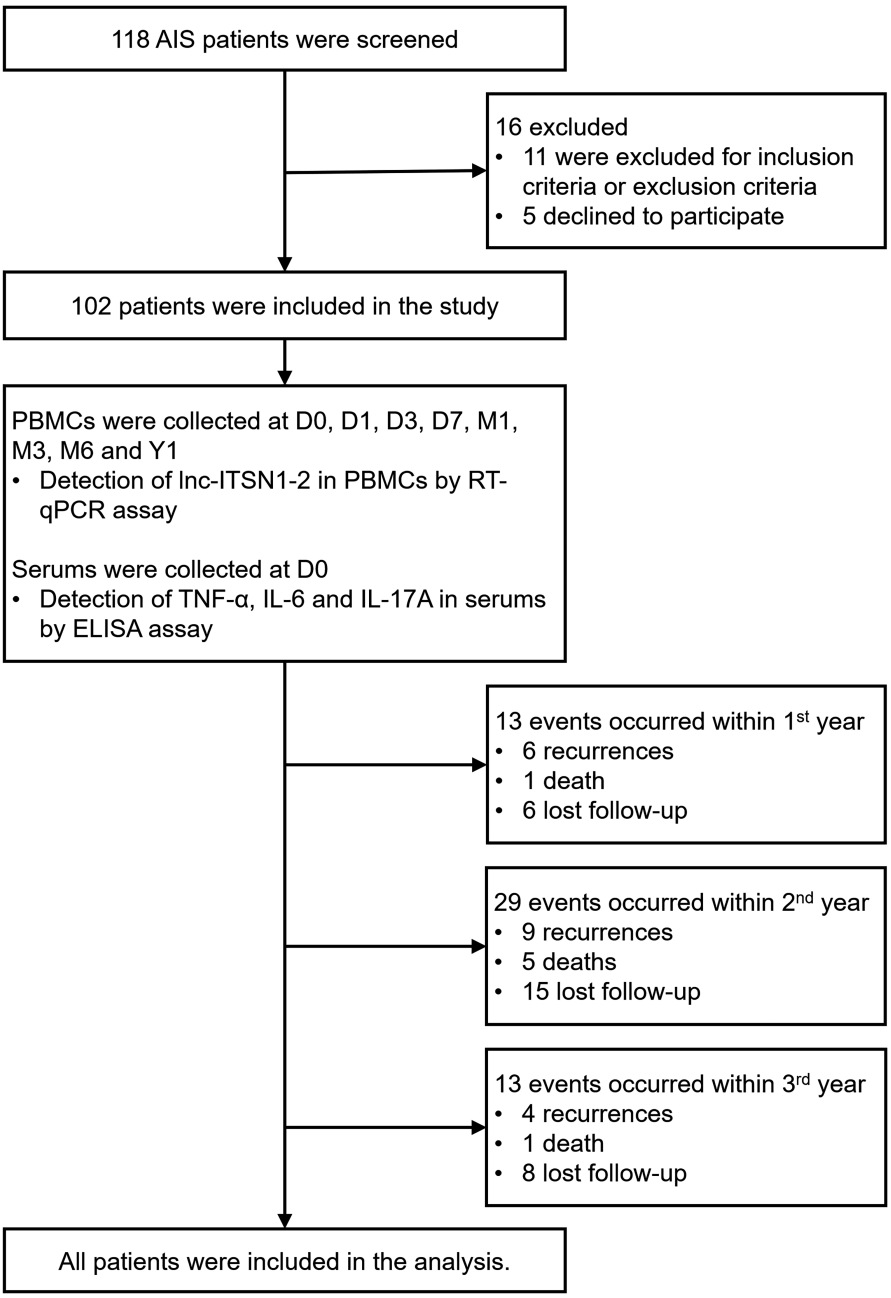


FIGURE 1 Study flow

serums were also collected at D0 in order to detect TNF-α, IL-6, and IL-17A by ELISA assay. During the follow-up duration, 13 events occurred within the 1st year (including 6 recurrences, 1 death, and 6 lost follow-up); 29 events occurred within the 2nd year (including 9 recurrences, 5 deaths, and 15 lost follow-up); 13 events occurred within the 3rd year (including 4 recurrences, 1 death, and 8 lost follow-ups). Lastly, all data from 102 patients were included in the analysis.

3.2 | Characteristics of patients and controls

The mean age of 102 AIS patients and 50 controls in this study were 65.2 ± 8.8 years and 65.6 ± 8.0, correspondingly. Besides, AIS patients were consisted of 29 (28.4%) females and 73 (71.6%) males; controls were consisted of 18 (36.0%) females and 32 (64.0%) males. As to the medical history of AIS patients, 52 (51.0%), 83 (81.4%), 36 (35.3%), 20 (19.6%), and 20 (19.6%) patients were also recognized with hyperlipidemia, hypertension, hyperuricemia, diabetes mellitus, and CKD, respectively. Moreover, the median National Institute Health of Stroke Scale (NIHSS) score of AIS patients was 6.5 (interquartile range (IQR): 4.0–10.0). Notably, all characteristics were of no difference between AIS patients and controls (all $p > 0.050$). Furthermore, the specific features of AIS patients were exhibited in Table 1.

3.3 | Expression of lnc-ITSN1-2 at baseline

Lnc-ITSN1-2 at baseline was up-regulated in AIS patients (median: 2.620 (IQR: 2.045–3.950)) than that in controls (median: 1.015 (IQR: 0.683–1.538); $p < 0.001$, Figure 2A). Additionally, lnc-ITSN1-2 at baseline showed good value to differentiate AIS patients from controls (area under the curve (AUC): 0.927, 95% confidence interval (CI): 0.888–0.966, Figure 2B).

TABLE 1 Characteristics of AIS patients and controls

Items	AIS patients ($N = 102$)	Controls ($N = 50$)	p value
Age (years), mean ± SD	65.2 ± 8.8	65.6 ± 8.0	0.801
Gender, *n* (%)			
Female	29 (28.4)	18 (36.0)	0.343
Male	73 (71.6)	32 (64.0)	
BMI (kg/m^2), mean ± SD	24.1 ± 2.6	23.5 ± 2.8	0.157
History of smoke, No. (%)			
No	40 (39.2)	27 (54.0)	0.085
Yes	62 (60.8)	23 (46.0)	
Hypertension, No. (%)			
No	19 (18.6)	9 (18.0)	0.925
Yes	83 (81.4)	41 (82.0)	
Hyperlipidemia, No. (%)			
No	50 (49.0)	21 (42.0)	0.415
Yes	52 (51.0)	29 (58.0)	
Hyperuricemia, No. (%)			
No	66 (64.7)	40 (80.0)	0.054
Yes	36 (35.3)	10 (20.0)	
Diabetes mellitus, No. (%)			
No	82 (80.4)	41 (82.0)	0.813
Yes	20 (19.6)	9 (18.0)	
CKD, No. (%)			
No	82 (80.4)	44 (88.0)	0.242
Yes	20 (19.6)	6 (12.0)	
NIHSS score, median (IQR)	6.5 (4.0–10.0)	-	-
TNF-α (pg/ml), median (IQR)	71.6 (54.1–103.0)	-	-
IL–6 (pg/ml), median (IQR)	38.4 (28.6–64.0)	-	-
IL–17A (pg/ml), median (IQR)	58.9 (49.9–70.8)	-	-

Abbreviations: AIS, acute ischemic stroke; BMI, body mass index; CKD, chronic kidney disease; IL-6, interleukin-6; IL-17A, interleukin-17A; IQR, interquartile range; NIHSS, National Institute Health of Stroke Scale; SD, standard deviation; TNF-α, tumor necrosis factor-alpha.

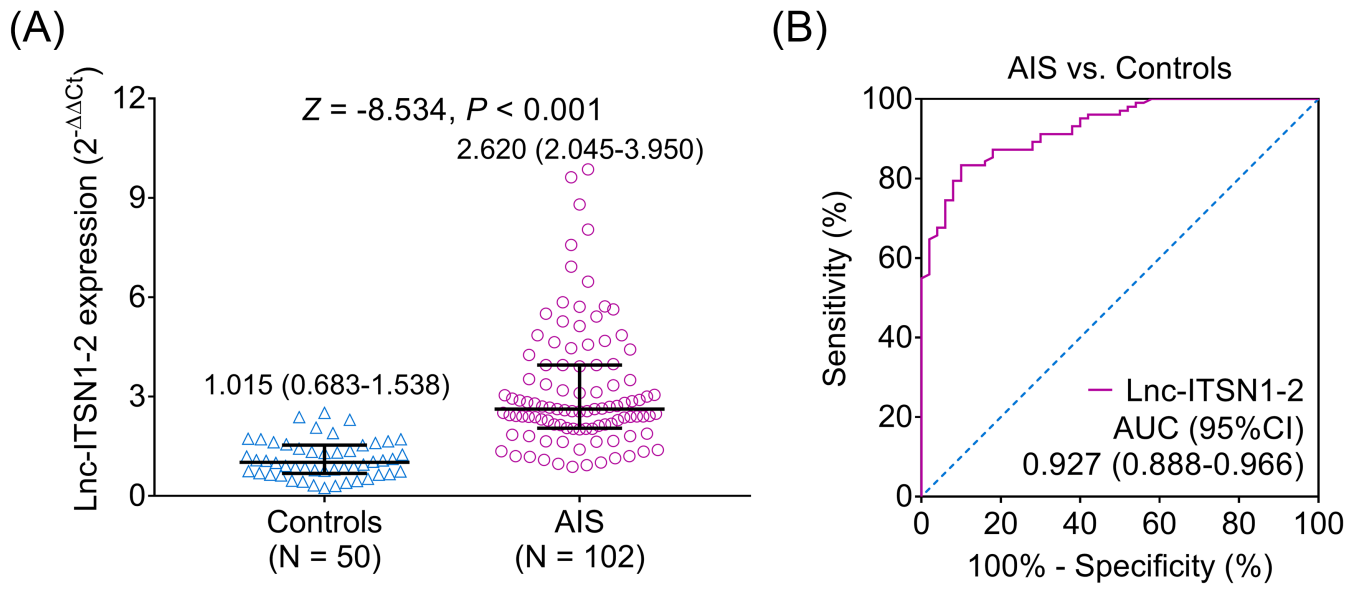


FIGURE 2 Lnc-ITSN1-2 was higher in acute ischemic stroke (AIS) patients than that in controls. Different expressions of lnc-ITSN1-2 in AIS patients and controls (A). The value of lnc-ITSN1-2 in distinguishing AIS patients from controls (B)

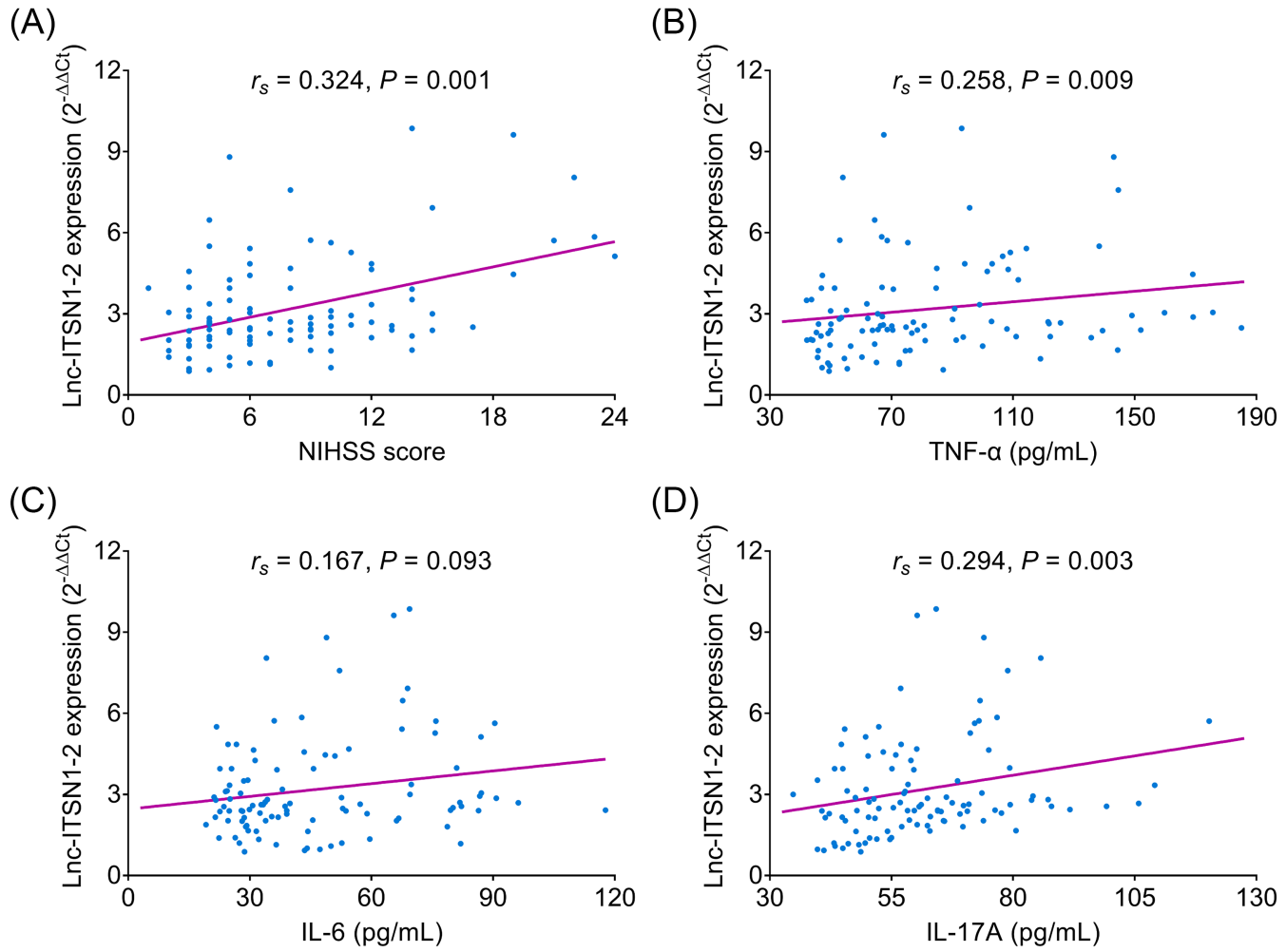


FIGURE 3 Up-regulated lnc-ITSN1-2 linked with high NIHSS score, TNF-α, and IL-17A in acute ischemic stroke (AIS) patients. Correlation of lnc-ITSN1-2 with NIHSS score (A), TNF-α (B), IL-6 (C), and IL-17A (D) in AIS patients

TABLE 2 Correlation of lnc-ITSN1-2 with medical history in AIS patients

Items	Lnc-ITSN1-2, median (IQR)	Statistic (*Z*)	*p* value
Hypertension			
No	2.420 (1.810–2.790)	−1.629	0.103
Yes	2.690 (2.120–4.420)		
Hyperlipidemia			
No	2.645 (1.658–3.625)	−0.633	0.527
Yes	2.620 (2.160–4.380)		
Hyperuricemia			
No	2.655 (2.175–3.920)	−0.399	0.690
Yes	2.550 (1.858–4.340)		
Diabetes mellitus			
No	2.495 (2.030–3.508)	−1.715	0.086
Yes	3.080 (2.598–4.450)		
CKD			
No	2.605 (2.045–3.920)	−0.426	0.670
Yes	2.790 (2.038–4.505)		

Abbreviations: AIS, acute ischemic stroke; CKD, chronic kidney disease; IQR, interquartile range; Lnc-ITSN1-2, long noncoding RNA intersectin 1–2.

3.4 | Linkage of lnc-ITSN1-2 at baseline with NIHSS score, inflammatory cytokines, and medical history

In AIS patients, up-regulated lnc-ITSN1-2 at baseline was related to increased NIHSS score (r_s = 0.324, p = 0.001), TNF-α (r_s = 0.258, p = 0.009), and IL-17A (r_s = 0.294, p = 0.003), while lnc-ITSN1-2 at baseline was not linked with IL-6 (r_s = 0.167, p = 0.093; Figure 3A-D). Besides, lnc-ITSN1-2 at baseline was not related to hyperlipidemia (p = 0.527), hypertension (p = 0.103), hyperuricemia (p = 0.690), diabetes mellitus (p = 0.086), or CKD (p = 0.670) in AIS patients (Table 2).

3.5 | Longitudinal change in lnc-ITSN1-2

Lnc-ITSN1-2 showed a dynamic variation during the 1-year follow-up period in AIS patients (p < 0.001, Figure 4). Specifically, lnc-ITSN1-2 was gradually increased from D0 (median: 2.620 (IQR: 2.045–3.950)) to D3 (median: 3.615 (IQR: 2.285–5.495)), while it switched to decrease from D3 to Y1 (median: 1.660 (IQR: 1.070–2.295)).

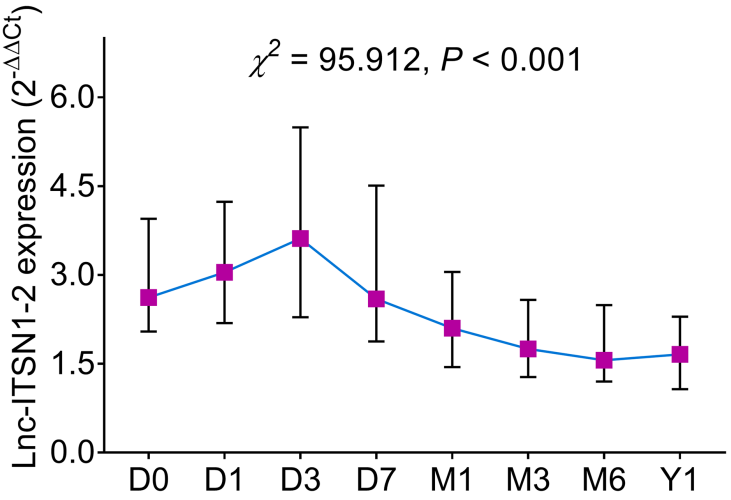
3.6 | Long-term prognosis

Acute ischemic stroke patients were continuously followed up at regular time intervals, with the median follow-up duration of 23.0 months (IQR: 17.0–30.3 months), ranging from 5.0 to 39.0 months. Until the last follow-up date of April 2021, 19 patients relapsed and 7 patients died with the accumulating recurrence rate of 18.6% (Figure 5A) and the accumulating mortality of 6.9% (Figure 5B).

3.7 | Linkage of lnc-ITSN1-2 with recurrence and death

Lnc-ITSN1-2 disclosed a similar variation trend in non-recurrent (p < 0.001, Figure 6A), recurrent (p = 0.001, Figure 6B), and survived (p < 0.001, Figure 6C) patients, which gradually increased from D0 to D3 but declined from D3 to Y1; while the variation of lnc-ITSN1-2 in died patients was not obvious (p = 0.132, Figure 6D). Notably, lnc-ITSN1-2 at D0 (p = 0.029), D3 (p = 0.017), D7 (p = 0.002), M1 (p < 0.001), M3 (p = 0.002), M6 (p = 0.010), and Y1 (p = 0.003) was up-regulated in recurrent AIS patients than that in non-recurrent AIS patients (Figure 6E). Besides, lnc-ITSN1-2 at D3 (p = 0.042), D7 (p = 0.014), M1 (p = 0.013), M3 (p = 0.018), and M6 (p = 0.032) was up-regulated in died patients than survived patients (Figure 6F).

For further verifying the correlation of lnc-ITSN1-2 with the recurrence and mortality of AIS, the univariate logistic regression



	No. Assessed patients	Lnc-ITSN1-2, median (IQR)
D0	102	2.620 (2.045-3.950)
D1	102	3.045 (2.188-4.238)
D3	94	3.615 (2.285-5.495)
D7	92	2.595 (1.875-4.510)
M1	85	2.100 (1.445-3.050)
M3	85	1.750 (1.275-2.580)
M6	79	1.560 (1.200-2.490)
Y1	73	1.660 (1.070-2.295)

FIGURE 4 Lnc-ITSN1-2 increased first, then gradually decreased in acute ischemic stroke (AIS) patients

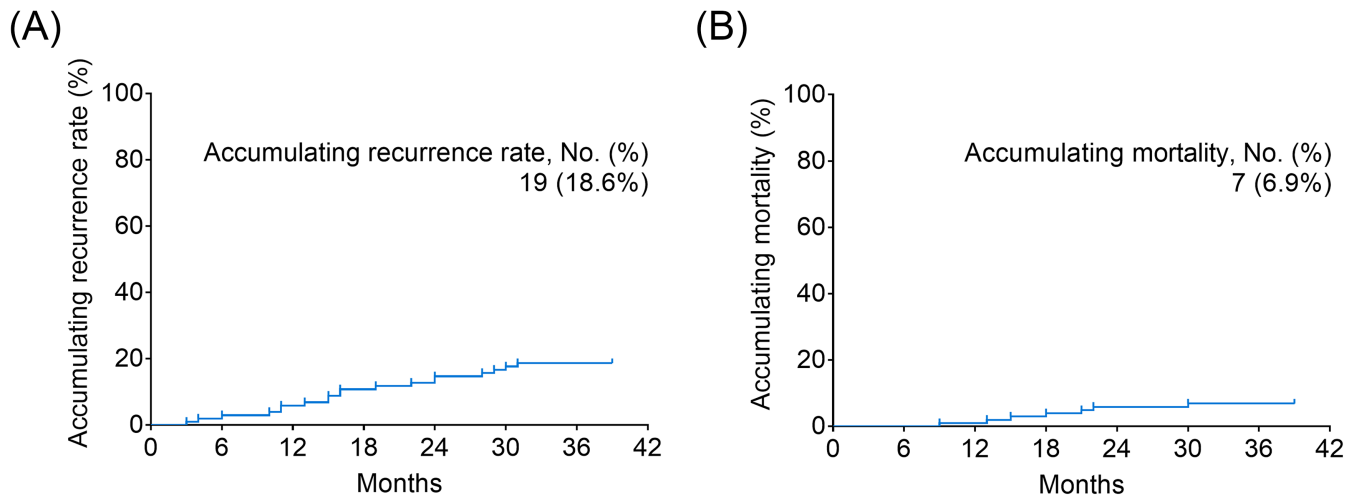


FIGURE 5 Recurrence and survival situation of acute ischemic stroke (AIS) patients. The accumulating recurrence rate (A) and mortality (B) of AIS patients within 42 months were 18.6% and 6.9%, respectively

analysis was conducted, which showed that higher lnc-ITSN1-2 at D0, D1, D3, D7, M1, M3, M6, and Y1 were associated with elevated recurrence risk in AIS patients (all $p < 0.050$; Table S1). Besides, higher lnc-ITSN1-2 at D1, D3, D7, M1, M3, M6, and Y1 were related to increased mortality risk in AIS patients (all $p < 0.050$; Table S2).

4 | DISCUSSION

ITSNs have been recognized as crucial regulators in several cellular processes (such as membrane trafficking processes, cytoskeletal remodeling, transcription, translation, and metabolic processes).[21–23] For example, one previous study shows that ITSN-1s dysregulation is responsible for pro-inflammatory endothelial-cell dysfunction induced by lipopolysaccharide.[21] Although sparse studies focus on lnc-ITSN1-2, its role in regulating inflammation has been noticed in several inflammatory disorders (including rheumatoid arthritis (RA), AS, SAP, and inflammatory bowel disease (IBD)).[18,20,24] For instance, one study observes that lnc-ITSN1-2 knockdown suppresses inflammation in the fibroblast-like synoviocytes of RA patients.[20] Considering that both neuroinflammation and systemic inflammation aggravate brain-tissue injury in AIS patients, it is speculated that lnc-ITSN1-2 might be associated with inflammatory cytokines and serve as a harmful factor in AIS.[7] This study found that lnc-ITSN1-2 was positively associated with NIHSS score, TNF-α, and IL-17A in AIS patients, which was similar to the previous study.[18] The probable explanations might be as follows: (1) Lnc-ITSN1-2 promoted inflammation and neuronal impairment, which increased cerebral injury in AIS patients.[13,15,25,26] Hence, increased lnc-ITSN1-2 was related to elevated NIHSS score in AIS patients. (2) Lnc-ITSN1-2 activated several pro-inflammatory pathways (including TNF pathway and nuclear-factor-kappa-B (NF-κB) pathway), then the secretions of inflammatory cytokines (such as TNF-α and IL-17A) were correspondingly promoted.[18] As a result, increased lnc-ITSN1-2 was related to elevated TNF-α and IL-17A in AIS patients.

At present, few study is conducted to investigate the longitudinal change in lnc-ITSN1-2 in AIS patients, while the current study noticed that lnc-ITSN1-2 was gradually increased from D0 to D3; while it switched to decrease from D3 to Y1 in AIS patients; also, it showed similar variations in recurrent, non-recurrent, died, and survived patients. The possible explanation might be that stroke injury had a hysteresis effect because AIS patients were easily suffered from ischemia-reperfusion injury and secondary brain damages after endovascular thrombectomy, which meant that the stroke injury of AIS patients aggravated initially, then remitted along with the revascularization.[27,28] Besides, considering the positive relationship between lnc-ITSN1-2 and disease severity in AIS patients, thus, lnc-ITSN1-2 was gradually increased from D0 to D3 but switched to decrease from D3 to Y1 in AIS patients.

As to the association of lnc-ITSN1-2 with recurrence and death in AIS patients, this study discovered that lnc-ITSN1-2 at most time points (including D0, D3, D7, M1, M3, M6, and Y1) was up-regulated in recurrent AIS patients than that in non-recurrent AIS patients; also, lnc-ITSN1-2 at D3, D7, M1, M3, and M6 was up-regulated in died patients than survived patients, which implied that patients with high lnc-ITSN1-2 during the recovery period might undergo elevated recurrence and death risk than patients with low lnc-ITSN1-2. The possible explanations might be that (1) T-helper (Th)1 cells could form into atherosclerotic plaques in the artery wall, which was associated with recurrent acute stroke.[29,30] Additionally, lnc-ITSN1-2 promoted the differentiation of $CD4^+$ T cells into Th1 cells via sponging microRNA-125a.[24] Hence, lnc-ITSN1-2 was positively associated with recurrence risk in AIS patients. (2) Lnc-ITSN1-2 was positively related to NIHSS score which was elevated in recurrence/died AIS patients.[18] Thus, lnc-ITSN1-2 at most time points was increased in recurrent patients (vs. non-recurrent patients) and died patients (vs. survived patients).

Some limitations were observed in this study. Firstly, this study set specific time points to collect samples, while not all patients followed up strictly on schedule. Thus, the number of patients who assessed lnc-ITSN1-2 decreased over time, which might interfere

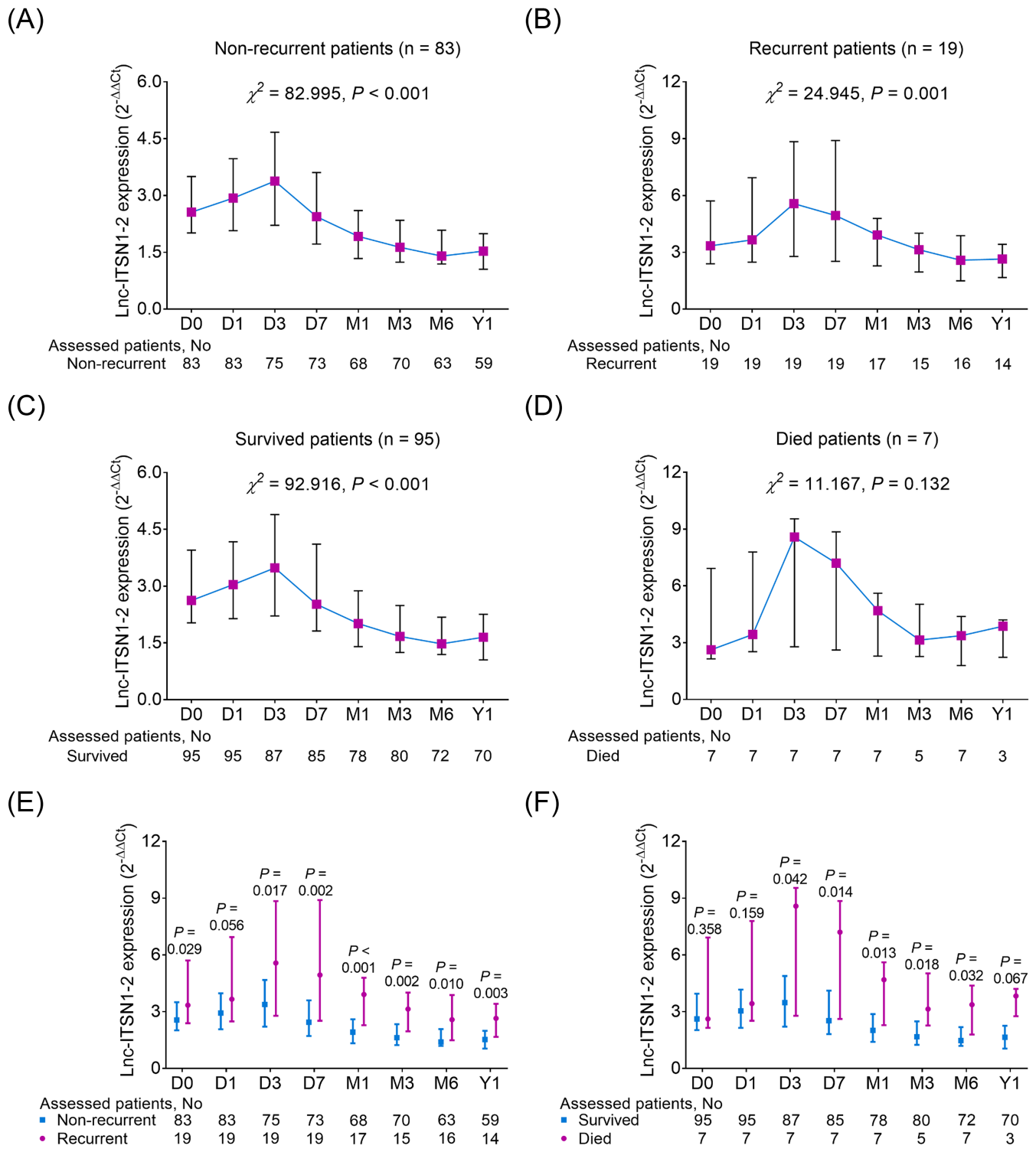


FIGURE 6 Lnc-ITSN1-2 at most time points were elevated in recurrent (vs. non-recurrent) and died (vs. survived) acute ischemic stroke (AIS) patients. The longitudinal changes (during 1-year follow-up) of lnc-ITSN1-2 in non-recurrent (A), recurrent (B), survived (C), and died (D) AIS patients. Comparison of lnc-ITSN1-2 at D0, D1, D3, D7, M1, M3, M6, and Y1 in recurrent vs. non-recurrent patients (E) and died vs. survived patients (F)

with the outcomes of the study. Secondly, the follow-up period was relatively short, while AIS patients needed long-time attention; thus, studies with longer follow-up periods were required. Thirdly, it was reported that lnc-ITSN1-2 was linked with Th cells in some inflammation-related disorders; hence, the association of lnc-ITSN1-2 with Th cells deserved further study in AIS patients. Fourthly, the current study only detected the relative expression of lnc-ITSN1-2 using RT-qPCR, while its absolute value was undetermined.

In conclusion, the dynamic variation of lnc-ITSN1-2 could serve as a biomarker in reflecting disease severity, inflammatory cytokines, recurrence, and death risk in AIS patients.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

ORCID

Qian Xue https://orcid.org/0000-0001-9945-0447

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Wang G, Zhou Y, Zhong T, Song A, Xue Q. Longitudinal variation of circulating lnc-ITSN1-2: A novel biomarker reflecting disease severity, inflammation, recurrence, and death risk in acute ischemic stroke patients. *J Clin Lab Anal*. 2022;36:e24468. doi:10.1002/jcla.24468