Neurology Asia 2025; 30(3) : 651 – 655

Different clinical classifications of ischemic stroke may lead to different conclusions

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Abstract

Objectives: To compare the differences in three ischemic stroke classification systems and their impact on the conclusions of gene polymorphism. *Methods:* Prospective analysis of inpatient data of acute ischemic stroke at Wenzhou Central Hospital from May 2022 to December 2022. Patients were classified using the Trial of Org 10172 in acute stroke treatment (TOAST) classification, Korean modified TOAST (KM TOAST) classification, and Chinese ischemic stroke subclassification (CISS). The PAI-1 4G/5G, eNOS G894T, ACE gene I/D and MTHFR Gene C677T loci were detected. *Results:* A total of 305 patients were included in this study. In TOAST, KM TOAST, and CISS systems, large-artery atherosclerosis (LAA) subtype accounted for 34.4%, 59.4% and 41.9% correspondingly, small-artery occlusion (SAO) subtype accounted for 37.4%, 21.3% and 26.2% respectively. And the lowest proportion was other definite cause (ODC) (1.3%, 1.3%, 1.3%). There were significant differences among the etiology subtypes of LAA, SAO, and Stroke of undetermined cause (UND). In the four gene loci detected, the genotype distribution of the PAI-1 4G/5G locus was different among the three etiological classification systems.
Conclusions: There are differences in the distribution of subtypes in the three ischemic stroke classification systems. The different etiological classification may have an impact on the conclusion of gene polymorphisms.

Keywords: Ischemic stroke, clinical classifications, gene polymorphisms, Chinese Ischemic Stroke Subclassification, Trial of Org 10172 in Acute Stroke Treatment, Korean modified TOAST

INTRODUCTION

The etiological classification of stroke is an indispensable part of stroke research. The correct clinical classification of acute ischemic stroke is important to the acute treatment of patients, secondary prevention and stroke-related research such as clinical trials, epidemiology and genetic studies. At present, the most widely used causative system is the Trial of Org 10172 in acute stroke treatment (TOAST) classification[1], and other stroke classification standards include Korean modified TOAST (KM-TOAST) classification[2], Chinese ischemic stroke subclassification (CISS)[3], ASCO classification[4] and SSS-TOAST.[5] There are some differences among various classification standards, which may lead to different research conclusions. In this study, TOAST system, KM-TOAST system and CISS system were carried out on the same group of patients, and the influence of different classification systems on the conclusion of gene polymorphism analysis was compared.

METHOD

Study population

The data of patients with acute ischemic stroke (onset≤7 days) who were hospitalized in the Neurology Department of Wenzhou Central Hospital from May 2022 to December 2022 were collected prospectively. Ischemic stroke was confirmed based on strict neurological examination, computer tomography and/or magnetic resonance imaging scan.

All patients underwent a comprehensive medical history, physical examination and clinical chemistry analysis. Risk factors of stroke were recorded, including age, gender, hypertension, diabetes mellitus, smoking,

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Date of Submission: 7 March 2025; Date of Acceptance: 30 June 2025
https://doi.org/10.54029/2025nps

drinking, dyslipidemia. The diagnostic criteria of the risk factors is the same as described in our previous study.

Excluded criteria included patients with (1) severe systemic or chronic disease, for example, hepatic cirrhosis and renal failure, (2) recurrent stroke patients, (3) incomplete clinical data.

Subtypes classification

Two neurologists classified all of the patients independently using TOAST KM-TOAST and CISS criteria. In patients with dissonant results, both the authors re-checked the data together before asserting the final diagnosis.

The TOAST system mainly based on clinical manifestations, imaging data and auxiliary examinations, ischemic stroke is divided into large-artery atherosclerosis (LAA), cardiogenic embolism (Cardioembolism, CE), and small-artery occlusion (SAO), other definite cause (Stroke of other determined cause, ODC), unknown cause (Stroke of undetermined cause, UND) 5 subtypes.

The KM-TOAST system includes five subtypes of brain infarction similar to those of the TOAST classification. It is divided into 5 subtypes: atherosclerotic thrombosis (atherothrombosis, AT), cardiogenic embolism (cardioembolism, CE), small vessel disease (small artery disease, SAD), other causes of stroke (stroke of other determined etiology, SOD), stroke of undetermined etiology (SUD). AT with significant stenosis of a large artery (ASLA) is a subtype of AT.

The CISS system includes large artery atherosclerosis (LAA), cardiogenic stroke (CS), penetrating artery disease (PAD), other etiologies (OE) and etiology unknown (undetermined etiology, UE).

Genotyping of relevant genes

Related gene detection was carried out for patients with TOAST LAA/SAO subtypes, KM-TOAST AT/SAD subtypes, and CISS LAA/PAD subtypes.

DNA extraction

Genomic DNA was extracted from peripheral venous blood samples using a DNA extraction kit (Sangon Biotech Co., Ltd. Shanghai, China) according to the manufacturer's instructions. All DNA samples were at -80°C until use.

Genetic polymorphism analysis

Genotypes of the PAI-1 4G/5G and the eNOS G894T polymorphisms were determined by TaqMan technology.[6,7] The ACE gene I/D polymorphism was studied using polymerase chain reaction as described elsewhere.[8] The MTHFR Gene C677T Polymorphism was genotyped by using polymerase chain reaction-restriction fragment length polymorphism assays.[9]

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics (Version 20.0; IBM Corporation, Armonk, NY, USA). Data on quantitative characteristics were expressed as the means±standard deviation. Qualitative data were presented as numbers and percentages. The association between categorical variables was assessed using the $\chi 2$ test, and if 20% of the cells had the expected count of lower than 5, the Fisher exact test was employed. For all inferential statistics, two-tailed P values less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

The first-ever ischemic stroke subjects included in this study were 194 men (63.6%) and 111 women (36.39%). The mean age was 67.76±13.26 years. Demographic, risk factor, and investigation profiles of the cohort are presented in Table 1. All patients had at least one brain imaging study (brain CT in 97.7%, MRI 91.8%, both in 87.9%). ECG and Angiographic studies were undertaken in both 305 patients (CTA in 64.9%, MRA in 88.5%, both in 15.8%). Transthoracic echocardiography was performed in 299 patients.

Frequency distribution of different etiological subtypes

Overall, the etiological subtypes of LAA and SAO accounted for a relatively high proportion of the three etiological classification systems (Table 2). LAA subtype accounted for 34.43%, 59.35% and 41.97%, SAO subtype accounted for 37.38%, 21.31% and 26.23% respectively, and the lowest proportion was ODC (1.31%, 1.31%, 1.31%). For UND subtype, among the three etiological classification systems, the CISS system had the highest proportion (76 cases, 24.92%), and The KM-TOAST system had the lowest proportion (38cases, 12.46%). There were significant differences among the etiology subtypes of LAA, SAO, and UND ($P<0.01$ for each).

Table 1: Demographics, clinical characteristics, and risk factors of patients

Demographics and clinical characteristics	Patients(n=305)
Age, years, mean ± SD	67.76 ± 13.26
Female, n(%)	111(36.39)
NIHSS at onset, median (range)	5(0-25)
Risk factors, n(%)	
Hypertension	250(81.97)
Diabetes mellitus	129(42.30)
Smoking	67(21.97)
Atrial fibrillation	17(5.57)
Diagnostic evaluation, n(%)	
Brain imaging	305(100)
CTA/MRA	305(100)
ECG	305(100)
Transthoracic echocardiography	299(98.03)

Abbreviations: NIHSS, national institute of health stroke scale; CTA, computed tomography angiography; MRA, magnetic resonance angiography; ECG, electrocardiogram.

The influence of different etiological classifications on the conclusion of genetic testing

Among the four gene loci detected, the conclusions of three loci were consistent among the three etiological classifications. However, the genotype distribution of the PAI-1 4G/5G locus was different among the three etiological classification systems. Different from TOAST and CISS, KM-TOAST suggested that the PAI-1 4G/5G genotype was a risk factor for LAA subtype (Table 3).

DISCUSSION

Our study compared the difference between TOAST system, KM-TOAST system and CISS system in patients with acute ischemic stroke. And discussed the influence of three etiological classifications on the conclusion of gene polymorphism analysis.

TOAST system is currently the most widely used etiological classification method, but the reliability between different reviewers is not high, and the proportion of "unknown cause" subtype is relatively large.[10] With the development of diagnostic techniques, the etiology can be determined in patients with multiple stroke mechanisms in the "unknown cause" subtype.[11-12] However, due to limited conditions when the TOAST classification was proposed, it was believed that as long as the diameter of the lesion was <1.5cm and the corresponding large artery stenosis was <50%; it was classified as SAO subtype, resulting in an excessively high proportion of this subtype. In addition, the TOAST classification is too strict in the definition of the etiology of large artery atherosclerosis, requiring that the corresponding large artery stenosis must be >50%, and the diameter of the lesion must be >1.5cm. This makes infarcts <1.5 cm in diameter, with >50% stenosis of the corresponding carrier artery, and other similar conditions can only be classified as unexplained etiologies.

Table 2: Subtypes distribution frequency with TOAST, KM-TOAST, and CISS

Subtype	TOAST, n(%)	KM TOAST, n(%)	CISS, n(%)	TOAST vs KM TOAST		TOAST vs CISS		KM TOAST vs CISS	
				χ^2	*P*	χ^2	*P*	χ^2	*P*
LAA/AT/LAA	105(34.43)	181(59.35)	128(41.97)	38.023	0.000	3.674	0.055	18.423	0.000
SAO/SAD/PAD	114(37.38)	65(21.31)	80(26.23)	18.984	0.000	8.738	0.003	2.036	0.154
CE/CE/CS	19(6.23)	17(5.57)	17(5.57)	0.118	0.731	0.118	0.731	0.000	1.000
UND/SUD/UE	63(20.65)	38(12.46)	76(24.92)	7.416	0.007	1.575	0.209	15.578	0.000
ODC/SOD/OE	4(1.31)	4(1.31)	4(1.31)	0.000	1.000	0.000	1.000	0.000	1.000

Table 3: The impact of TOAST, KM-TOAST and CISS on the conclusion of genetic testing

Gene	Genotype	TOAST		χ^2	*P*	KM TOAST		χ^2	*P*	CISS		χ^2	*P*
		LAA	SAO			AT	SAD			LAA	PAD		
ACE	I/I	54	50	1.257	0.533	89	25	3.679	0.159	62	32	2.392	0.302
	I/D	40	50			73	28			52	34		
	D/D	11	14			19	12			14	14		
MTHFR	C/C	33	32	0.965	0.617	58	20	0.528	0.769	42	25	0.095	0.954
	C/T	54	66			95	37			66	43		
	T/T	18	16			28	8			20	12		
PAI	4G/4G	20	33	3.215	0.200	34	23	7.688	0.021	25	24	4.094	0.129
	4G/5G	61	55			108	29			78	38		
	5G/5G	24	26			39	13			25	18		
eNOS	T/T	1	0	1.137	0.566	1	0	3.245	0.197	1	0	0.803	0.669
	G/T	10	10			16	8			12	9		
	G/G	94	104			164	57			115	71		

In 2007, Korean scholars proposed an improved TOAST classification. Patients with systemic atherosclerosis were classified as AT subtype. The size or location of the lesion is not a consideration. It can be seen that the application of KM-TOAST typing reduces the missed diagnosis rate of AT subtype, and also reduces the proportion of SUD subtype.[13]

The CISS classification is currently widely used in China. CISS classifies aortic arch atherosclerosis as the cause of large artery atherosclerosis rather than as cardioembolic stroke. Vulnerable plaque or stenosis ≥ 50% is regarded as evidence of corresponding intracranial or extracranial large artery atherosclerosis. With evidence of vulnerable plaques or stenosis ≥50% in ipsilateral proximal intracranial or extracranial large arteries, isolated penetrating artery infarct is classified in undetermined etiology (UE; multiple etiology).

In this study, there were significant differences in the subtypes of LAA, SAO, and UND among the three etiological classification systems. The proportion of LAA subtypes in KM-TOAST classification was significantly higher than that in CISS and TOAST typing. Compared with TOAST classification, the proportion of SAO subtype in KM-TOAST and CISS classifications was significantly lower.

Most ischemic strokes are caused by a combination of multiple susceptibility genes and environmental factors. The research on the candidate genes of cerebral infarction is the focus of the research on the genetic mechanism of cerebral infarction.[14,15] The genetic basis of different types of cerebral infarction is different.[16-19] Heritability varied markedly by stroke subtype being 40.3% for large-vessel disease and 32.6% for cardioembolic but lower for small-vessel disease (16.1%).[20]

In this study, the single nucleotide polymorphism sites of four genes in the same batch of ischemic stroke patients were detected, and the three different etiological typing methods were used. The results showed that 1/4 of the genotyping had different conclusions. The findings suggest that different etiological types may have an impact on the conclusion of gene polymorphisms. Consistency analysis of different etiological classification systems is urgently needed, so that the data between different research centers can be compared and used to promote the development of ischemic stroke research.

There are some limitations in our research: Firstly, as a Single-center clinical research, selection bias is inevitable. Secondly, our study sample was relatively small. In addition, due to the lack of continuous electrocardiogram monitoring, we may have missed some patients with cardiogenic embolism stroke. Further large-scale studies are necessary to investigate the impact of different etiological classification systems on genetic research.

ACKNOWLEDGEMENT

We thank the patients for their consent to participate in this study.

DISCLOSURE

Ethics: The study was approved by the Ethics Committee of Wenzhou Central Hospital, in compliance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Financial support: This work was supported by funding from the Wenzhou Science & Technology Bureau (NO.Y20210908, NO.Y20200718), Panvascular Disease Management Center, Wenzhou Central Hospital (NO.2022HZSY0045).

Conflict of interests: None

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