The Association Between Genetic Variants in lncRNA-p53 Regulatory Network and Ischemic Stroke Prognosis

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Research

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Abstract

Objective Recently, lncRNAs have been demonstrated to be associated with the p53 regulatory pathway, which serve as regulators or effectors. lncRNA-p53 regulatory network plays an important role in ischemia-induced apoptosis and is important for post-stroke recovery.

Method Eight genetic variants in p53-related lncRNA were genotyped in 940 patients to explore the association of SNPs in p53 regulatory pathway genes with ischemic stroke prognosis in a northern Chinese population. Long-term outcome and short-term outcome were respectively assessed by the stroke recurrence and modified Rankin Scale score at 3 months after stroke.

Results We first identified that p53 rs1042522 and LINC-ROR rs2027701 may be associated with the risk of IS recurrence. In our further cumulative effect analysis, we found that these two polymorphisms may jointly be associated with IS recurrence. Patients carrying 2–4 risk alleles had significantly increased risk of IS recurrence compared with those carrying 0–1 risk alleles. In contrast to rs2027701 and rs1042522, other SNPs were found to be not associated with IS recurrence. Subsequently, we also found that TUG1 rs2240183 CC genotype was associated with favorable outcome of ischemic stroke after adjusting confound factors. However, the other seven genetic variants in p53-related lncRNA were not associated with functional outcome after stroke.

Conclusion P53 rs1042522 and LINC-ROR rs2027701 may have combined effects on IS recurrence and TUG1 rs2240183 may be a new biomarker to predict the short-term outcome of ischemic stroke through modulating p53-mediated apoptosis.

Introduction

Ischemic stroke (IS) making up 2/3 stroke is main mortality and disability in China, bring heavy financial and mental burden to society and family [1]. Thorough understanding about the pathophysiology of stroke is necessary. Functional recovery after stroke is decided by post-stroke neuronal damage, which are related by pathophysiological process. Currently, it is reported that the mechanisms of post-stroke injury include inflammation, apoptosis of penumbra, excitotoxicity and oxidative stress [2–3]. Neuron apoptosis is main reason for penumbra damage [2–3]. Identification the effect of different genetic background on apoptosis would provide a new path for IS prognosis.

Long noncoding RNAs (lncRNAs) are a class of more than 200 nucleotides RNA that barely involved in protein coding but regulating gene expression at post-transcriptional and transcriptional levels, epigenetic regulation, chromatin modification [4]. Our previous review have indicated that the lncRNAs expression profile was altered in MCAO animal models and blood of IS patients [5]. lncRNAs has found to be play vital roles in pathophysiological processes of IS and post-stroke recovery by regulating apoptosis [6–7]. P53 regulatory network participate in the process of cellular apoptosis associated pathophysiology of post-stroke by regulating the expression of many downstream genes [8–9]. Recently, lncRNAs have been demonstrated to be associated with the p53 regulatory pathway, as shown in Fig. 1. On the one hand,

lncRNAs such as Plasmacytoma variant translocation 1 (PVT1), metastasis associated lung adenocarcinoma transcript 1 (MALAT1), murine double minute 2 (MDM2) could act as p53 regulators by controlling p53 stability [10–11]. In addition, lncRNAs are involved in the sophisticated regulatory system concerning the maintenance of p53 stability. On the other hand, the expression levels of lncRNAs pR-lncRNA-1, lncRNA-P21, LINC-PINT, Taurine Upregulated Gene 1 (TUG1) and Regulator of reprogramming (LINC-ROR) are regulated by p53 and are p53 effectors [12–16]. In our study, we highlight several lncRNAs related to the p53 pathway and determine whether lncRNA-p53 regulatory network SNPs are associated with prognosis of IS.

Previous studies in this field are only focused on few SNPs in coding genes. A thorough investigation for the association of genetic variation within lncRNA-p53 regulatory pathway with IS prognosis in Chinese population remains lacking. In our study, we plan to explore the association of SNPs in lncRNA-p53 regulatory pathway genes with IS prognosis in a northern Chinese population. Furthermore, we investigate the gene-gene interaction in lncRNA-p53 regulatory pathway, which could provide more insights into the genetic background for prognosis of stroke. Our study aimed to identify lncRNA-p53 regulatory pathway genetic biomarkers for prediction of the recurrent risk of IS and functional outcome of IS patients.

Method

Study subjects

This study contains first-ever IS cases from the First affiliated hospital of China Medical University between November 2016 and December 2019. IS were diagnosed on the basis of both clinical features and diffusion weighted imaging (DWI) scan. The inclusion and exclusion criteria of patients are the same to our previous study [17]. Our study was approved by Ethics Committee of the First Hospital of China Medical University and in accordance with the principles stated in the Declaration of Helsinki. All the patients signed the informed consent. National Institute of Health stroke scale (NIHSS) score was assessed on admission to evaluate stroke severity for all the patients. All the patients were followed up through telephone interview or clinical visit every three months until recurrence. Hypertension, diabetes mellitus, dyslipidemia, drinking and smoking were defined as described in our previous study [17]. Functional outcome was assessed with modified Rankin Scale (mRS) after 3 months. Patients with mRS score equal to or more than 2 defined as unfavorable outcome and patients with mRS 0–1 defined as favorable outcome. Patients who had died after their enrollment were assigned to poor outcome group.

SNP Selection

In our study, SNPs of p53 related lncRNA-gene were selected using dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP>) and lncRNA-SNP database (<http://bioinfo.life.hust.edu.cn/lncRNASNP/>). The minor allele frequency for these SNPs should be exceeding 5% and r^2 was

less than 0.8. SNP function prediction was performed using HaploReg 4.1 (<http://www.broadinstitute.org/mammals/haploreg>).According to the above criteria,p53 rs1042522;MDM2 rs937283; LincRNA-P21 rs4713998;LINC-ROR rs2027701;pR-lncRNA-1 rs3743773; LINC-PINT rs1059698;TUG1 rs2240183 and PVT1 rs13281615 were selected in our study. The detail of SNP was shown in the Table 1. Genomic DNA was extracted from blood using the DNA Blood Mini Kit.These SNPs were genotyped by PCR-ligase detection reaction method (PCR-LDR),which was described in previous study[17].

Table 1
, Detailed information of the 8 SNPs

Gene	SNP	SNP Location	Alleles	1000G-CHBS
P53	rs1042522	Chr17:7676154 – 7579472	C/T	0.5745
MDM2	rs937283	chr12:68808384–69202164	A/G	0.2524
LincRNA-P21	rs4713998	chr6:36664833 – 36632610	A/G	0.2187
LINC ROR	rs2027701	chr18:57057964 – 54725195	A/G	0.4254
pR-lncRNA-1	rs3743773	chr16:53044095–53078007	G/A	0.2355
LINC-PINT	rs1059698	chr7:130944335 – 130629094	A/C	0.3100
TUG1	rs2240183	chr22:30966153–31362139	C/T	0.6394
PVT1	rs13281615	chr8:127343372–128355618	A/G	0.4783

Statistical analysis

Categorical variables were compared using Chi-square test and continuous variables using independent t-test.The association between lncRNA SNP and short-term outcome was accessed under the additive, dominant, and recessive model using the chi-square test. Logistic regression was used to evaluated the associations between lncRNA SNP and short-term outcome after adjusting for confound factors. The association between lncRNA SNP and stroke recurrence were accessed using cox proportional hazards regression model and Kaplan–Meier survival .The combined effect of SNP–SNP interactions was accessed according to the number of risk allele. All the analyses were performed with SPSS 21.0 and P < 0.05 was considered as statistical significant.

Results

Basic clinical characteristics of enrolled patients

42 patients (4.5%) did not finish the follow up, and eventually 940 patients were included in our study for stroke recurrence analysis.Among them, 139 patients had recurrent stroke. The median follow-up time

was 14 months. The age and stroke subtype was associated with stroke recurrence, and diabetes mellitus have a trend to be risk for stroke recurrence (Table 2).

Table 2
Clinical characteristics of patients grouped by long-term prognosis

Variables	Patients N = 940 (%)	Recurrence N = 139	Log-rank p
Age	358	42	0.019
≤ 55	582	97	0.78
> 55	634	94	0.096
Sex	306	45	0.286
Male	560	76	0.024
Female	380	63	0.132
Diabetes	551	84	0.918
No	589	55	0.536
Yes	527	91	
Smoking	413	48	
No	211	26	
Yes	729	113	
TOAST	574	83	
LAA	366	56	
SVD	724	108	
Hypertension	216	31	
No			
Yes			
Hyperlipidemia			
No			
Yes			
Drinking			
No			
YES			

P53 Related Lncrnas Polymorphisms And Ischemic Stroke Recurrence

p53 rs1042522 was associated with ischemic stroke recurrence risk under dominant model (95%CI = 1.099–2.527, $p = 0.016$, OR = 1.666) not under recessive model (95%CI = 0.727–1.643, $p = 0.669$, OR = 1.093). Subjects who carrying CG + GG have higher IS recurrence risk. We also identified that LINC-ROR rs2027701 was related with IS recurrence risk. The GG genotype of LINC-ROR rs2027701 was increased the risk of ischemic stroke recurrence under recessive model (95%CI = 1.070–2.311, $p = 0.021$, OR = 1.573). In contrast to rs2027701 and rs1042522, other SNP rs937283, rs4713998, rs3743773, rs1059698, rs2240183 and rs13281615 was not associated with IS recurrence risk. (Table 3–4, Fig. 2).

Table 3
The association between p53 related lncRNA and IS
recurrence

Genotype	All the cases		
	Patients	Recurrence	Log-rank p
rs1042522	268	28	0.01
CC	492	81	0.063
CG	180	30	0.137
GG	454	74	0.923
rs1059698	404	51	0.137
AA	82	14	0.923
AC	238	36	0.932
CC	458	72	0.028
rs13281615	244	31	0.275
AA	319	42	0.513
AG	455	62	0.248
GG	166	31	0.523
rs2027701	305	39	0.248
AA	479	77	0.523
AG	156	23	0.991
GG	608	96	0.426
rs2240183	300	40	
TT	32	3	
CT	671	102	
CC	251	32	
rs3743773	18	5	
GG	480	68	
GA	382	52	
AA	78	13	
rs4713998			
AA			

Genotype	All the cases
AG	
GG	
rs937283	
AA	
AG	
GG	

Table 4
,Association between p53 rs1042522 ,LINC ROR rs2027701 and IS recurrence

Genotype of SNP	Patients	Recurrence	<i>p</i>	HR	(95% CI)a
rs1042522	268	28	0.016	1.666	1.099–2.527
CC	492	81	0.669	1.093	0.727–1.643
CG	180	30	0.418	1.163	0.807–1.674
GG	111/672	28/268	0.021	1.573	1.070–2.311
Dominant model	109/760	30/180			
CG + GG VSCC	319	42			
Recessive model	455	62			
GG VS CG + CC	166	35			
rs2027701	97/621	42/319			
AA	35/166	104/774			
AG					
GG					
Dominant model					
GG + AG VS AA					
Recessive model					
GG VS AA + AG					

Cumulative Effect Of Rs1042522-rs2027701 On Is Recurrence

Based on the above findings, we explored the cumulative effect of lncRNA-p53 regulatory pathway SNPs on IS recurrence risk. The combined effects of the two polymorphisms was accessed according to the number of risk alleles. Patients were classified into two groups according to the number of risk alleles (rs2027701 G and rs1042522 G allele). Patients with 2–4 risk alleles had 1.732-fold (95%CI = 1.188–2.500, $p = 0.004$) increased risk of IS recurrence relative to those with 0–1 risk alleles. Our results showed that subjects carrying more than 1 risk allele had higher recurrence risk of IS than patients with 0–1 risk allele (Table 5).

Table 5
Combined analyses of p53 rs1042522, LINC ROR rs2027701 and IS Recurrence

Number of risk alleles	Patients	Recurrence	<i>p</i>	HR	(95% CI) ^a
0–1	378	39	0.004	1.732	1.188–2.500
2–4	562	100			
Risk alleles are defined as rs1042522 G and rs2027701 G alleles.					

Stratification analyses for combined effect of p53 related lncRNAs on functional outcome of stroke

According to the above evidence, stratified analysis by general standards was conducted to estimate the associations between the combined SNP polymorphisms and stroke recurrence risk. The higher recurrence risk was more obvious among subgroups with male ($p = 0.015$, OR = 1.756, 95%CI = 1.114–2.767), older ($p = 0.017$, OR = 1.748, 95% CI = 1.105–2.764), no hypertension ($p = 0.003$, OR = 6.488, 95% CI = 1.143–21.985), diabetes ($p = 0.016$, OR = 2.091, 95%CI = 1.149–3.085), non-drinking ($p = 0.01$, OR = 1.740, 95%CI = 1.143–2.648), hyperlipidemia ($p = 0.006$, OR = 2.428, 95% CI = 1.293–4.589) (Table 6).

Table 6

Stratified analysis of p53 rs1042522-LINC ROR rs2027701 associated with recurrence of ischemic stroke

Variable	Genotype (recurrence/patients)		P a	HR (95% CI)a
	0–2 variants	3–4 variants		
Total	14/149	28/209	0.128	1.655(0.866–3.163)
Age	25/229	72/353	0.017	1.748(1.105–2.764)
<=60	13/127	32/179	0.114	1.689(0.883–3.237)
> 60	26/251	68/383	0.015	1.756(1.114–2.767)
Sex	25/237	51/323	0.084	1.529(0.945–2.476)
Male	14/141	49/239	0.016	2.091(1.149–3.085)
Female	22/232	62/319	0.05	2.01(1.233–3.277)
Diabetes	17/146	38/243	0.318	1.343(0.753–2.394)
No	17/168	31/245	0.632	1.158(0.635–2.112)
Yes	22/210	69/317	0.020	2.183(1.346–3.539)
Smoking	3/85	23/126	0.003	6.488(1.195–21.985)
No	36/293	77/436	0.124	1.367(0.918–2.038)
Yes	26/233	57/341	0.129	1.436(0.900–2.290)
TOAST	13/145	43/221	0.006	2.428(1.293–4.589)
SVD	31/298	77/426	0.01	1.74(1.143–2.648)
LAA	8/80	23/136	0.114	1.926(0.854–4.347)
Hypertension				
No				
Yes				
Hyperlipidemia				
No				
Yes				
Drinking				
No				
YES				

Fig,2 Kaplan-Meier survival curves for recurrence among ischemic stroke (A.Dominant mode of rs1042522 ; B,Recessive model of rs2027701; C.Combined effect of rs1042522- rs2027701)

p53 related lncRNAs polymorphisms and short-term outcome of ischemic stroke

A total of 878 patients were included for this analysis of short outcome of IS. The characteristics of enrolled patients are presented in Table 7. 456 patients (51.9%) had a poor outcome (mRS of 2–6) and 422 patients (48.1%) had a poor outcome (mRS of 0–1). Patients with poor outcomes are more likely to older ($P = 0.021$), diabetes and subject with a higher NIHSS score on admission ($P < 0.001$), as shown in Table 7.

Table 7
Clinical characteristics of patients grouped by short-term prognosis

Variable	MRS(0–1) N = 422	MRS(2–5) N = 456	<i>p</i>
Age ≥ 60	229	307	0.000
Male	298	296	0.071
Hypertension	324	358	0.538
Diabetes	149	198	0.014
Hyperlipidemia	168	172	0.525
Smoking	180	188	0.669
Drinking	97	105	0.989
NIHSS	3.161.398	8.243.641	0.000
TOAST	180	303	0.000

Association between lncRNA-p53 regulatory pathway and post-stroke recovery was accessed for each SNP with regard to functional outcome after 3 months. The CC genotype of SNP rs2240183 was associated with favorable functional outcome 3 months after IS (Table 8). Furthermore, this correlation was still observed after adjustment for age, sex, smoking, drinking, hypertension, NIHSS score and diabetes (Table 9). However, other p53 related lncRNA snps showed no association with functional outcome after 3 months. (Table 8)

Table 8
, p53 related lncRNA and their association with IS short-term outcome

SNP	MRS(0–1) N = 422	MRS(2–5) N = 456	<i>p</i>	<i>OR</i>	95%CI
rs1042522	124	134	Reference	1.070	0.788–1.453
CC	211	244	0.664	0.830	0.561–1.227
CG	87	78	0.349	1.000	0.748–1.337
GG	298/124	322/134	0.999	0.795	0.566–1.115
Dominant model	87/335	78/378	0.183	1.154	0.874–1.524
CG + GG VS CC	211	211	Reference	1.194	0.738–1.934
Receesive model	175	202	0.312	1.161	0.891–1.514
GG VS CG + CC	36	43	0.470	1.116	0.702–1.775
rs1059698	211/211	245/211	0.269	0.981	0.709–1.358
AA	36/386	43/413	0.642	0.858	0.593–1.243
AC	104	118	Reference	0.937	0.691–1.271
CC	203	226	0.909	0.896	0.642–1.176
Dominant model	115	112	0.419	1.067	0.794–1.434
CC + AC VS AA	318/104	338/118	0.675	1.075	0.727–1.588
Receesive model	115/307	112/344	0.363	1.069	0.890–1.414
CC VS AC + AA	147	152	Reference	1.034	0.729–1.468
rs13281615	203	224	0.667	1.127	0.838–1.517
AA	72	80	0.718	0.704	0.472–1.050
AG	275/147	304/152	0.639	1.003	0.757–1.328
GG	72/350	80/376	0.85	0.655	0.458–0.937
Dominant model	140	151	Reference	0.948	0.713–1.262
GG + AG VS AA	199	242	0.428	0.782	0.366–1.674
Receesive model	83	63	0.085	0.932	0.707–1.229
GG VS AG + AA	282/142	305/151	0.985	0.796	0.374–1.694
rs2027701	83/339	63/393	0.02	1.229	0.911–1.695
AA	140	151	Reference	1.645	0.591–4.528
AG	199	242	0.716	1.252	0.934–1.678

SNP	MRS(0-1) N = 422	MRS(2-5) N = 456	p	OR	95%CI
GG	83	63	0.526	1.555	0.56-4.315
Dominant model	153/269	158/298	0.619	1.112	0.842-1.468
GG + AG VS AA	15/407	13/443	0.553	1.427	0.864-2.357
Recessive model	310	314	Reference	1.159	0.889-1.511
GG VS AG + AA	106	132	0.177	1.360	0.837-2.212
rs2240183	6	10	0.336		
TT	112/310	142/314	0.133		
CT	6/416	10/446	0.393		
CC	221	222	Reference		
Dominant model	171	191	0.454		
CC + CT VS TT	30	43	0.164		
Recessive model	201/221	234/222	0.275		
CC VS CT + TT	30/392	43/413	0.213		
rs3743773					
GG					
GA					
AA					
Dominant model					
AA + GA VS GG					
Recessive model					
AA VS GA + GG					
rs4713998					
AA					
AG					
GG					
Dominant model					
GG + GA VS AA					
Recessive model					

SNP	MRS(0–1) N = 422	MRS(2–5) N = 456	<i>p</i>	<i>OR</i>	95%CI
GG VS AG + AA					
rs937283					
AA					
AG					
GG					
Dominant model					
GG + GA VS AA					
Recessive model					
GG VS AG + AA					

Table 9
Ischemic stroke short outcome prognosis factors in the logistic regression analysis

	<i>p</i>	<i>OR</i>	95%CI
Sex	0.381	0.795	0.475–1.329
Age	0.513	1.155	0.751–1.776
Hypertension	0.692	1.108	0.668–1.838
Diabetes	0.861	1.040	0.673–1.605
Hypercholesterolemia	0.314	0.801	0.519–1.235
Smoking	0.823	0.994	0.570–1.564
Drinking	0.132	0.638	0.355–1.144
NIHSS	0.000	2.892	2.495–3.352
Genotype CC of rs2240183	0.014	0.499	0.285–0.871

Discussion

In our study, we explored the association of all SNPs in lncRNA-p53 regulatory network with IS prognosis in a total of 940 northern Chinese. We first identified that p53 rs1042522 and LINC-ROR rs2027701 may single and synergistically correlate with the risk of IS recurrence. Subsequently, we found that TUG1 rs2240183 CC genotype was associated with favorable outcome of ischemic stroke. However, the other seven genetic variants in p53 related lncRNA were not associated with functional outcome after stroke. To our knowledge, this is the first study to explore the lncRNA-p53 regulatory network genetic variation

with short and long outcome of ischemic stroke. It also provides a novel therapeutic target for brain ischemia and opens up a new perspective to the genetic background for stroke prognosis.

Neuron apoptosis is the main mechanism involved in penumbra, the border of ischemic core where the neuron injury is reversible. Recent studies have found several lncRNAs are involved in p53-related apoptosis pathway. Some lncRNAs can act as effectors and p53 transcriptional targets in the p53 pathway. For example, lncRNA-p21 was one of p53-regulated lncRNA and can regulate the transcriptional activity of p53 by influencing the interaction between p53, p300 and MDM2[18]. Additionally, lncRNA-p21 decreased the p53-MDM2 interaction and increased p53-p300 interaction by directly binding to MDM2 which thereby enhances apoptosis[19]. Another study was shown that the lncRNA *Pint* was a direct p53 target and could be motivated after p53 activation[12]. Over-expressing *Pint* could attenuate cellular apoptosis and increase cell proliferation. Furthermore, lncRNA Taurine Upregulated Gene 1 (TUG1) was a direct transcriptional target of p53 through interaction with the putative p53 response element and increased neuronal apoptosis as p53 effector[16]. Other lncRNAs can serve as master gene regulators in the p53 signaling pathway. Plasmacytoma variant translocation 1 (PVT1) is found to decrease apoptosis by partaking in the MDM2-p53 pathway, which could stabilize MDM2 protein expression and inhibit p53 expression[13]. Regulator of reprogramming (lncRNA-*RoR*) not only influences p53 protein levels as p53 regulator but also *under* p53 transcriptional control as effector[11]. In addition, pR-lncRNA-1 is not only directly regulate p53 transcriptional activity, but also induced by p53. pR-lncRNA-1 was also found to be decreased apoptosis regulate binding of p53 to its targets[15]. p53 is the major mediator of apoptosis-related pathophysiology process after stroke. The above lncRNAs have been found to be linked with the p53 regulatory pathway. Activation of lncRNA-p53 pathway can trigger apoptosis under hypoxia and ischemia, pro-apoptotic proteins such as (Bcl-2, PUMA) is releasing. Identification of p53-related lncRNAs pathway and mediating ischemia-induced apoptosis is a promising therapeutic strategy for saving the penumbra and minimizing the infarct volume.

p53-mediated neuronal apoptosis plays a vital role on stroke pathophysiology and ischemic penumbra injury. Until now, two studies have investigated the p53 apoptotic pathway-related gene (*P53*, *P21*, *MDM-2*, and *MMP-9*) with functional outcome after IS. Cristina et al. has first found that MDM2 rs2279744 G allele had increased MDM2 protein expression and was associated with better functional outcome after stroke[21]. Their results revealed that MDM2 rs2279744 determines the functional outcome of patients after stroke through regulating the MDM2-p53 interaction. Subsequently, a study by Yi et al. identified that interactions among P53 rs1042522, MDM-2 rs2279744, and MMP-9 rs3918242 may jointly increase the risk of poor functional outcome and may be a biomarker of predicting poor functional outcome after stroke[22]. However, the role of p53 apoptotic pathway-related lncRNA genes polymorphism and IS prognosis has not been thoroughly understood.

In our study, we first identified for that P53 rs1042522 and LINC-ROR rs2027701 may correlate with the risk of IS recurrence. In our further cumulative effect analysis, we found that these two polymorphisms may jointly be associated with IS recurrence. Patients carrying 2–4 risk alleles had significantly increased

risk of IS recurrence compared with those carrying 0–1 risk alleles. Our study indicated that LINC-ROR-p53 interaction plays a role in the prognosis of IS. The IS recurrence risk was identified to be enhanced with the increasing number of risk allele of two SNPs. The higher recurrence risk is more evidence among subgroups with male, older, diabetes, hyperlipidemia. The LINC-ROR-p53 interaction participate in the pathophysiology of IS through regulating neuron apoptosis. In addition, the 1-kb sequence upstream of regulator of reprogramming (ROR) is a p53-binding site that induces ROR expression. ROR expression suppresses p53 to maintain cellular homeostasis [11]. Gao et al. demonstrate that LINC-ROR regulated p53 ubiquitination and modulated cell apoptosis. Silence LINC-ROR inhibited the expression of p53 and its downstream target gene [23]. LINC-ROR-p53 interaction could synergistically induced activation of apoptotic pathway and lead to brain injury. Therefore, our investigation uncovered a novel mechanism by which p53 and LINC-ROR regulate each other's expression and activity in a feedback manner. This effect may also contribute to the higher IS recurrence risk of patients with the GG genotype. It is pity that we fail to find the association between the other six genetic variants and recurrent risk of IS.

In the following study, we found that CT + TT genotype of TUG1 rs2240183 was associated with poor outcome of ischemic stroke. We also found that the patients with CT + TT of rs2240183 were statistically associated with an increased risk of poor outcome of IS compared to CC genotype after covariate adjustment. Previous study has reported that TUG1 is over-expressed in cortical neurons of MCAO mice and OGD model. Over-expressed TUG1 promotes neuronal apoptosis and induces deterioration of neurological function [23]. In the previous report, it has been shown that rs2240183 is located in the promoter of lncRNA TUG, which interferes with its production and mature process by binding to GATA-1, and the C allele of rs2240183 could influence expression of the mature lncRNA TUG [24]. Additionally, SNPs located in lncRNA could affect miRNA-lncRNA interactions, eventually influence the expression levels of lncRNA. We speculated that rs2240183 may alter TUG1 structure and expression level [25]. The above evidence indicated that TUG1 rs2240183 gene might be a candidate gene for stroke prognosis since it may influence expression of TUG1, eventually regulating apoptosis and neuron injury. However, the other seven variants were not significantly associated with functional outcome of post-stroke.

Most studies have demonstrated only 1 or 2 candidate genes related to post-stroke recovery, but our studies have focus on lncRNA-p53 regulatory network which give us a comprehensive perspective of stroke prognosis genetics. Gene–gene interaction also be emphasized and confer a higher risk than single variant. Whereas the risk allele of any one SNP in the lncRNA-p53 pathway may only have a modest influence on IS progression, it is obvious that joint results of risk alleles has the potential to contribute significant. However, there are some limits in our study. First, p53 pathways are very complex, and many genetic variants may participated in regulating apoptosis. Thus, a larger set of genetic variants must be explored to uncover the full map of gene-gene interaction effect on functional outcome in the further study. Second, we did not conduct the experiment to reveal the molecular mechanisms of the gene-gene interactions.

Conclusion

LINC-ROR-p53 interaction play a role in brain injury and apoptosis and have combined effect on stroke recurrence. Our study also indicates that TUG rs2240183 is associated with favorable functional outcome 3-months post-stroke. In our study, we revealed that gene-gene interaction of lncRNA-p53 regulatory network may influence stroke recovery and significantly affect post-stroke apoptosis. This pathway may provide a novel genetic biomarker with predictive values for the clinical outcome of IS patients. The underlying mechanisms that mediate penumbra cell death are needed to deeply investigate in the future .

Abbreviations

Ischemic stroke(IS);Long noncoding RNAs (lncRNAs)plasmacytoma variant translocation 1 (PVT1), metastasis associated lung adenocarcinoma transcript 1(MALAT1);murine double minute 2(MDM2),taurine Upregulated Gene 1(TUG1); Regulator of reprogramming(LINC-ROR);National Institute of Health stroke scale(NIHSS).

Declarations

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Availability of data and material

The data used in our study are available from the authors on reasonable request.

Authors' contributions

Designed the experiments: Ruixia ZhuPerformed the experiments: Ruixia Zhu, Xu Liu ,Jingjing zhaoAnalyzed

the data: Xu Liu ,Qianwen Wang,Hongtao Chang. Wrote the paper: Ruixia Zhu ,Qianwen Wang

Compliance with ethical standards and consent to participate

This study was approved by the ethics committee of the First Affiliated Hospital of China Medical University approval, in accordance with the principles of the Helsinki Declaration(AF-SOP-07-1.0-01). Written informed consents were obtained from all the participants.

Consent for publication

Not applicable.

Conflict of interest

The authors have no conflict of interests.

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Figures

Figure 1

LncRNAs associated with the p53 pathway.

Figure 2

Kaplan-Meier survival curves for recurrence among ischemic stroke patients. A. Dominant mode of rs1042522; B. Recessive model of rs2027701; C. Combined effect of rs1042522- rs2027701)