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

Xu Liu
The First Hospital of China Medical University: The First Affiliated Hospital of China Medical University

qianwen wang
The First Hospital of China Medical University: The First Affiliated Hospital of China Medical University

jingjing zhao
The First Hospital of China Medical University: The First Affiliated Hospital of China Medical University

hongtao chang
The First Hospital of China Medical University: The First Affiliated Hospital of China Medical University

ruixia zhu (✉️ zrx_200626313@163.com )
the first affiliated Hospital of China Medical University  https://orcid.org/0000-0002-2683-4674

Research

Keywords: ischemic stroke, p53 related IncRNAs, recurrence, functional outcome

DOI: https://doi.org/10.21203/rs.3.rs-126670/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Objective Recently, IncRNAs have been demonstrated to be associated with the p53 regulatory pathway, which serve as regulators or effectors. LncRNA-p53 regulatory network play important role in ischemia induced apoptosis and be importance for post-stroke recovery.

Method Eight genetic variants in p53 related IncRNA were genotype 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 SNP was 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 IncRNA 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 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 (IncRNAs) are a class of more than 200 nucleoties 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 IncRNAs 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, IncRNAs 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, LincRNA-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 SNP are associated with prognosis of IS.

Previous studies in this field are only focused on few SNP in coding genes. A thoroughly 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 contain first-ever IS cases from the 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 (MRI) 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 singed the informed consent. National Institute of Health stroke scale (NIHSS) score was assessed on admission to evaluate stroke severity for all the patents. All the patients were followed up through telephone interview or clinical visit every three month until recurrence. Hypertension, diabetes mellitus, dyslipidemia, drinking and smoking were defined as described in our previous study[17]. Functional outcome was accessed with modified Rankin Scale (mRS) after 3 months. Patients with mRS score equal to or more than 2 define as unfavorable outcome and patients with mRS 0–1 define 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 lncRNASNP 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].

| 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).

| Variables        | Patients N = 940 (%) | Recurrence N = 139 | Log-rank p |
|------------------|----------------------|--------------------|------------|
| Age ≤ 55         | 358                  | 42                 | 0.019      |
| Age > 55         | 582                  | 97                 | 0.78       |
| Sex Male         | 306                  | 45                 | 0.286      |
| Sex Female       | 560                  | 76                 | 0.024      |
| Diabetes No      | 380                  | 63                 | 0.132      |
| Diabetes Yes     | 551                  | 84                 | 0.918      |
| Smoking No       | 589                  | 55                 | 0.536      |
| Smoking Yes      | 527                  | 91                 |            |
| TOAST LAA        | 358                  | 42                 |            |
| TOAST SVD        | 582                  | 97                 |            |
| Hypertension No  | 634                  | 94                 | 0.096      |
| Hypertension Yes | 634                  | 94                 |            |
| Hyperlipidemia   | No                   | 211                | 0.286      |
| Hyperlipidemia   | Yes                  | 729                |            |
| Drinking No      | 211                  | 26                 |            |
| Drinking Yes     | 729                  | 113                |            |
| Drinking YES     | 216                  | 31                 |            |
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).
| 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 | p   | HR   | (95% CI)a |
|-----------------|----------|------------|-----|------|-----------|
| rs1042522       | 268      | 28         | 0.016 | 1.666 | 1.099–2.527 |
| CG              | 492      | 81         | 0.669 | 1.093 | 0.727–1.643  |
| GG              | 180      | 30         | 0.418 | 1.163 | 0.807–1.674  |
| Dominant model  | 111/672  | 28/268     | 0.021 | 1.573 | 1.070–2.311  |
| 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 IncRNA-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).

| Number of risk alleles | Patients | Recurrence | p   | HR   | (95% CI) |
|------------------------|----------|------------|-----|------|----------|
| 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 <=60 | 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) |
| Sex Male | 26/251                        | 68/383 | 0.015 | 1.756(1.114–2.767) |
| 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 No | 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 No | 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) |

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.

| Variable       | MRS(0–1) | MRS(2–5) | p     |
|----------------|----------|----------|-------|
|                | N = 422  | N = 456  |       |
| 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          | 3.16     | 3.64     | 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 | p         | OR     | 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     | 87/335           | 78/378           | 0.183     | 1.154  | 0.874–1.524 |
| recessive    | 211              | 211              | Reference | 1.194  | 0.738–1.934 |
| 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     | 115              | 112              | 0.419     | 1.067  | 0.794–1.434 |
| recessive    | 318/104          | 338/118          | 0.675     | 1.075  | 0.727–1.588 |
| rs13281615   | 147              | 152              | Reference | 1.034  | 0.729–1.468 |
| AA           | 203              | 224              | 0.667     | 1.127  | 0.838–1.517 |
| AG           | 72               | 80               | 0.718     | 0.704  | 0.472–1.050 |
| GG           | 275/147          | 304/152          | 0.639     | 1.003  | 0.757–1.328 |
| Dominant     | 72/350           | 80/376           | 0.85      | 0.655  | 0.458–0.937 |
| recessive    | 140              | 151              | Reference | 0.948  | 0.713–1.262 |
| rs2027701    | 199              | 242              | 0.428     | 0.782  | 0.366–1.674 |
| AA           | 83               | 63               | 0.085     | 0.932  | 0.707–1.229 |
| AG           | 282/142          | 305/151          | 0.985     | 0.796  | 0.374–1.694 |
| recessive    | 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) | MRS(2−5) | p     | OR    | 95%CI   |
|--------------|----------|----------|-------|-------|---------|
|              | N = 422  | N = 456  |       |       |         |
| 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 |
| Receesive 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 |       |         |
| Receesive 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 |        |          |       |       |         |
| Receesive model |        |          |       |       |         |
| AA VS GA + GG |        |          |       |       |         |
| rs4713998    | AA       | AG       | GG    |       |         |
| Dominant model |        |          |       |       |         |
| GG + GA VS AA |        |          |       |       |         |
| Receesive model |        |          |       |       |         |
| SNP                          | MRS(0–1) | MRS(2–5) | p   | OR   | 95% CI       |
|------------------------------|----------|----------|-----|------|--------------|
| N = 422                     | N = 456  |          |     |      |              |
| 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

|                                           | p   | OR   | 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 novel therapeutic targets for brain ischemia and opens up a new perspective to the genetic background for stroke prognosis.

Neuron apoptosis is the main mechanism involved in the penumbra, the border of the ischemic core where the neuron injury is reversible. Recent studies have found several lncRNAs are involved in p53-related apoptosis pathways. Some lncRNAs can act as effectors and p53 transcriptional targets in the p53 pathway. For example, LincRNA-p21 was one of p53-regulated lncRNA and can regulate the transcriptional activity of p53 by influencing the interaction between p53, p300 and MDM2. Additionally, LincRNA-p21 decreased the p53-MDM2 interaction and increased p53-p300 interaction by directly binding to MDM2 which thereby enhances apoptosis. Another study showed that the lncRNA Pint was a direct p53 target and could be activated after p53 activation. Over-expressing Pint could attenuate cellular apoptosis and increased 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. Other lncRNAs can serve as master gene regulators in the p53 signaling pathway. Plasmacytoma variant translocation 1 (PVT1) is found to decrease apoptosis by participating in the MDM2-p53 pathway, which could stabilize MDM2 protein expression and inhibiting p53 expression. Regulator of reprogramming (lncRNA-RoR) not only influences p53 protein levels as p53 regulator but also under p53 transcriptional control as effector. 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. 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. 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. 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 IncRNA 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 IncRNA TUG[24]. Additionally, SNPs located in IncRNA could affect miRNA-IncRNA interactions, eventually influence the expression levels of IncRNA. 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 IncRNA-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 IncRNA-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**

**Acknowledgments**

We are deeply grateful to all participants of this study.

**Funding**

This study was supported by Natural Science Foundation of Liaoning province of China (2019-MS-364) and the National Natural Science Foundation of China (81501006).

**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 Zhu.
Performed the experiments: Ruixia Zhu, Xu Liu, Jingjing Zhao.
Analyzed 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.

References

1. Liu L, Wang D, Wong KS, Wang Y. Stroke and stroke care in China: huge burden, significant workload, and a national priority. Stroke. 2011;42:3651–4.

2. Khoshnam SE, Winlow W, Farzaneh M. et al. Pathogenic mechanisms following ischemic stroke. Neurol Sci. 2017;38:1167–86.

3. Puyal J, Ginet V, Clarke PG. Multiple interacting cell death mechanisms in the mediation of excitotoxicity and ischemic brain damage: a challenge for neuroprotection. Prog Neurobiol. 2013;105:24–48.

4. Iyer MK, Niknafs YS, Malik R. et al. The landscape of long noncoding RNAs in the human transcriptome. Nat Genet. 2015;47:199–208.

5. Yang J, Zhao J, Liu X, Zhu R. LncRNAs a New Target for Post-Stroke Recovery. Curr Pharm Des. 2020;26(26):3115–21.

6. Bao MH, Szeto V, Yang BB. et al. Long non-coding RNAs in ischemic stroke. Cell Death Dis. 2018;15(3):281.. ;9.

7. Zhang X, Hamblin MH, Yin KJNoncoding. RNAs StrokeNeuroscientist. 2019;25(1):22–6.

8. Zhang A, Xu M, Mo YY. Role of the IncRNA-p53 regulatory network in cancer. J Mol Cell Biol. 2014;6:181–91.

9. Lin T, Hou PF, Meng S, Chen F, Jiang T, Li ML, Shi ML, Liu JJ, Zheng JN, Bai J. Emerging Roles of p53 Related IncRNAs in Cancer Progression: A Systematic Review. Int J Biol Sci. 2019;12(6):1287–98.. ;15.

10. Zhang T, Wang H, Li Q, Fu J, Huang J, Zhao YMALAT. 1 Activates the P53 Signaling Pathway by Regulating MDM2 to Promote Ischemic Stroke. Cellular Physiology and biochemistry. International Journal of Experimental Cellular Physiology Biochemistry Pharmacology. 2018;50(6):2216–28.

11. Guo J, Hao C, Wang C, Li L. Long noncoding RNA PVT1 modulates hepatocellular carcinoma cell proliferation and apoptosis by recruiting EZH2. Cancer Cell Int. 2018;11:18:98.

12. Marín-Béjar O, Marchese FP, Athie A, Sánchez Y, González J, Segura V, Huang L, Moreno I, Navarro A, Monzó M, García-Foncillas J, Rinn JL, Guo S, Huarte M. Pint lincRNA connects the p53 pathway with epigenetic silencing by the Polycomb repressive complex 2. Genome Biol. 2013;14(9):R104.

13. Zhang A, Zhou N, Huang J, Liu Q, Fukuda K, Ma D, Lu Z, Bai C, Watabe K, Mo YY. The human long non-coding RNA-RoR is a p53 repressor in response to DNA damageCell Res. 2013;23(3):340 – 50.

14. Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, KenzelmannBroz D, Khalil AM, Zuk O, Amit I, Rabani M, Attardi LD, Regev A, Lander ES, Jacks T, Rinn JL. A large intergenic noncoding RNA
induced by p53 mediates global gene repression in the p53 response. Cell. 2010;142:409–19.

15. Sánchez Y. et al. Genome-wide analysis of the human p53 transcriptional network unveils a lncRNA tumour suppressor signature. Nat Commun. 2014;5:812.

16. Zhang EB, Yin DD, Sun M, Kong R, Liu XH, You LH, Han L, Xia R, Wang KM, Yang JS, De W, Shu YQ. Wang ZX. P53-regulated long non-coding RNA TUG1 affects cell proliferation in human non-small cell lung cancer, partly through epigenetically regulating HOXB7 expression. Cell Death Dis. 2014, 22;5:e1243.

17. Liu X, Wang Q, Zhu R. Association of GWAS-susceptibility loci with ischemic stroke recurrence in a Han Chinese population. J Gene Med. 2020;25:e3264.

18. Zhang Y, Miao Y, Shang M, Liu M, Liu R, Pan E, Pu Y, Yin L. LincRNA-p21 leads to G1 arrest by p53 pathway in esophageal squamous cell carcinoma. Cancer Manag Res. 2019;4:11:6201–14.

19. Wu G, Cai J, Han Y, Chen J, Huang ZP, Chen C, Cai Y, Huang H, Yang Y, Liu Y, Xu Z, He D, Zhang X, Hu X, Pinello L, Zhong D, He F, Yuan GC, Wang DZ, Zeng C. LincRNA-p21 regulates neointima formation, vascular smooth muscle cell proliferation, apoptosis, and atherosclerosis by enhancing p53 activity. Circulation. 2014;21(17):1452–65.

20. Rodríguez C, Ramos-Araque ME, Domínguez-Martínez M, Sobrino T, Sánchez-Morán I, Agulla J, Delgado-Esteban M, Gómez-Sánchez JC, Bolaños JP, Castillo J, Almeida A. Single-Nucleotide Polymorphism 309T > G in the MDM2 Promoter Determines Functional Outcome After Stroke. Stroke. 2018, 49(10):2437–2444.

21. Yi X, Zhou Q, Sui G, Ren G, Tan L, Li J, Lin J, Bao S. Interactions among variants in P53 apoptotic pathway genes are associated with neurologic deterioration and functional outcome after acute ischemic stroke. Brain Behav. 2020;7:e01492.

22. Gao H, Wang T, Zhang P, Shang M, Gao Z, Yang F, Liu R. Linc-ROR regulates apoptosis in esophageal squamous cell carcinoma via modulation of p53 ubiquitination by targeting miR-204-5p/MDM2. J Cell Physiol. 2020;235(3):2325–35.

23. Xiong ZJ, Zhang Q, Wang DX, Hu L. Overexpression of TUG1 promotes neuronal death after cerebral infarction by regulating microRNA-9. Eur Rev Med Pharmacol Sci. 2018;22(21):7393–400.

24. Wei YS, Yang J, HeYL, Shi X, Zeng ZN. A functional polymorphism in the promoter of TUG1 is associated with an increased risk of ischaemic stroke. J Cell Mol Med. 2019;23(9):6173–81.

25. Yang C, Tang R, Ma X, Wang Y, Luo D, Xu Z, Zhu Y, Yang L. Tag SNPs in long non-coding RNA H19 contribute to susceptibility to gastric cancer in the Chinese Han population. Oncotarget. 2015;20(17):15311-20.

Figures
Figure 1

LncRNAs associated with the p53 pathway.

Figure 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)