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Serum CircNIPSNAP3A is Associated with Metabolic Disorders, Atherosclerosis and Severity of Coronary Artery Disease in a Chinese Population

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The relationships of serum circNIPSNAP3A and circHIPK3 with metabolic disorders, atherosclerosis and severity of coronary artery disease (CAD) remain to be clarified. Three hundred and thirty-eight subjects were categorized into normal coronary artery, atherosclerosis and CAD groups. Clinical data including anthropometric indexes, medical history, and physiological and biochemical parameters were collected. Serum circNIPSNAP3A and circHIPK3 were determined by quantitative real-time PCR. CAD severity was evaluated by clinical manifestation, electrocardiogram and coronary angiography. Both CAD and atherosclerosis groups had a higher serum level of circNIPSNAP3A than the normal coronary artery group ($P < 0.05$ for all). The subjects with a high percentage (> 66th percentile) of circNIPSNAP3A had higher mean levels of triglycerides, uric acid and homocysteine, and lower mean levels of high-density lipoprotein cholesterol and apolipoprotein AI than those with a low percentage (< 33rd percentile) of circNIPSNAP3A. Notably, circNIPSNAP3A is significantly and independently associated with CAD, and subjects with a high percentage of circNIPSNAP3A had more diseased coronary branches and a higher incidence of acute coronary syndrome than those with a low percentage of circNIPSNAP3A. Regarding circHIPK3, subjects with a medium or high percentage of circHIPK3 had a lower mean level of apolipoprotein AI than those with a low percentage of circHIPK3, but no significant differences in the incidence and severity of CAD among the < 33rd, 33rd-66th, and > 66th percentiles of circHIPK3 were detected. Serum circNIPSNAP3A is related to cardiovascular risk factors and CAD severity, and may be a potential prognostic marker and/or therapeutic target for CAD.

Keywords: atherosclerosis; circHIPK3; circNIPSNAP3A; coronary artery disease; serum
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Introduction

Coronary artery disease (CAD), a leading cause of death around the world, is a very complex disease that involves a range of genetic and non-genetic risk factors (Zhang et al. 2019; Walli-Attaei et al. 2020). Circular RNAs (circRNAs) are a novel class of endogenous non-coding RNAs with profound regulatory effects on gene expression and protein functions (Liu and Chen 2022; Liu et al. 2023; Wei et al. 2023). The vast majority of circRNAs come from gene exons, with a few originating from introns or both. CircRNAs are single-stranded, closed-loop RNA molecules, which are more stable compared to linear RNAs (Sun and Yang 2023). CircRNAs mainly act as a

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molecular sponge to adsorb miRNAs, thereby eliminating the inhibition of miRNAs to their target genes, and in turn enhancing the expression of the target genes (Meng et al. 2023; Wen et al. 2023). In addition, circRNAs can also exert regulatory effects through other ways, such as serving as templates for peptide translation (Mo et al. 2020; Song et al. 2023a), regulating the activity of RNA-binding proteins as ligands (Ikeda et al. 2023; Zhong et al. 2023), and combining with DNA to regulate gene expression or with RNA to regulate RNA splicing (Li et al. 2023). CircRNAs have been shown to be associated with atherosclerosis, and implicated in the occurrence and development of CAD (Azizidoost et al. 2023; Chen et al. 2023, Farina et al. 2023; Jiapaer et al. 2023; Yang and Rong 2023).

CircNIPSNAP3A (hsa_circ_0001879) is formed by back-splicing of the last exon and its downstream sequence of NipSnap homolog 3A gene (*NIPSNAP3A*), with a total length of 13,647 bp. CircNIPSNAP3A has been identified to be highly expressed in peripheral blood mononuclear cells of patients with CAD by microarray analysis (Wang et al. 2019). Li et al. (2021) found that circNIPSNAP3A is up-regulated in atherosclerotic plaques of CAD patients as well as in oxidized low-density lipoprotein (ox-LDL)-induced human umbilical vein endothelial cells (HUVECs). CircHIPK3 (hsa_circ_0000284) is a 1,099 bp long circRNA composed of a single exon, i.e., exon 2 of homeodomain interacting protein kinase 3 gene (*HIPK3*). Zhang et al. (2022) demonstrated that circHIPK3 is low expressed in arterial tissue and plasma of patients with atherosclerosis, and exerts a protective role for atherosclerosis via the miR-106a-5p/MFN2 axis. The anti-atherosclerotic effect of circHIPK3 may also be achieved by regulating the miR-637/CDK6 axis or by activating the autophagy flux (Wei et al. 2020; Kang et al. 2021).

Herein, a hospital-based study was conducted with angiographically defined atherosclerosis and CAD patients, as well as the subjects with normal coronary arteries to investigate the associations of serum levels of circNIPSNAP3A and circHIPK3 with cardiovascular risk factors, susceptibility and severity of CAD. The results of this study can provide an opportunity to unveil the interrelationships among serum circNIPSNAP3A and circHIPK3, cardiovascular risk factors, occurrence and development of CAD.

Subjects and Methods

Subjects

A total of 338 consecutive and unrelated Chinese adult subjects who underwent coronary angiography for suspected CAD at the Department of Cardiology, Affiliated Hospital of Chengdu University (Chengdu, China) were enrolled in the study between January 2022 and January 2023. The exclusion criteria were as follows: 1) patients with valvular disease, hepatic or renal dysfunction or inflammatory disease; 2) patients taking medication that may affect the levels of serum glucose, lipids, uric acid (UA) and homocysteine; 3) patients underwent percutaneous coronary intervention. The patients who were taking medicines that do not affect cardiovascular disease risk factors were still enrolled in the study to enlarge the sample size. All of the participants or their guardians provided signed informed consent prior to participation in the study. The study protocol was reviewed and approved by Ethics Committee of Chengdu University. The tenets of the Declaration of Helsinki were adhered to in all procedures reported in the article.

Definitions and measurements

Smoking was defined as regular cigarette smoking or having at least three years of smoking experience. Hypertension was defined as regular use of antihypertensive drugs or systolic/diastolic blood pressure ≥ 140/90 mmHg. Type 2 diabetes mellitus (T2DM) was defined as regular use of hypoglycemic drugs or fasting glucose level ≥ 7.0 mmol/L. Body mass index (BMI) was calculated by dividing weight by height squared (kg/m^2). Fasting blood samples were taken on the first morning after hospitalization of the patients when the drugs that may affect cardiovascular risk factors were not used yet. Serum was separated and used for the measurement of the following parameters: total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, lipoprotein (a) [Lp(a)], apolipoprotein B (APOB), apolipoprotein AI (APOAI), UA, glucose, cystatin c (CysC), homocysteine and hypersensitive C-reactive protein (hs-CRP). Enzymatic method was employed to measure the variables including TC, HDL-C, LDL-C, triglycerides, glucose, UA and homocysteine. Immunoturbidimetric assay was used to determine the variables including Lp(a), APOAI, APOB, CysC and hs-CRP. All tests were conducted by using an automatic clinical chemistry analyzer (Beckman Coulter AU5800, California, USA).

Serum RNA extraction and quantitative real-time polymerase chain reaction

Blood samples were collected from each subject. Serum specimens were isolated by centrifugation and stored at −80°C immediately. Long-chain RNAs (> 200 nt) were extracted by using TIANamp Virus RNA Kit (TIANGEN, Beijing, China) according to the instruction manual. One microgram of RNA was reverse-transcribed to cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific Fermentas, Waltham, Massachusetts, USA) according to the manufacturer's protocol. To detect the expression levels of circNIPSNAP3A and circHIPK3, the following quantitative PCR assay was performed using TB Green Premix Ex Taq II (Tli RNaseH Plus) (Takara, Osaka, Japan). Data were normalized using GAPDH as the internal reference and the relative expression was calculated by using the $2^{-\Delta\Delta CT}$ method. The differences among plates were normalized and corrected using standard cDNA samples. The forward and reverse primers used for circNIPSNAP3A

were 5′-TGTGGTTTATTTCTGCAGTTCATGT-3′ and 5′-AGGTAGTTGACACTTTCCCG-3′, respectively. The forward and reverse primers used for circHIPK3 were 5′-TTCAACATATCTACAATCTCGGT-3′ and 5′-ACCAT TCACATAGGTCCGT-3′, respectively. The forward and reverse primers used for GAPDH were 5′-TGGGTGTG AACCATGAGAAGT-3′ and 5′-TGAGTCCTTCCAC GATACCAA-3′, respectively.

CAD diagnosis and severity assessment

Coronary angiography was performed by two experienced cardiologists who did not have any information on the clinical characteristics of the patients with suspected CAD. Allura Xper FD20 (PHILIPS, Amsterdam, Netherlands) was employed to carry out coronary angiography with at least two views of right coronary arteries (RCA) and four views of the left coronary system including left main coronary artery (LM), left anterior descending artery (LAD) and left circumflex artery (LCX). The subjects with stenosis greater than 50% in any of the major coronary arteries were diagnosed as CAD, and those with coronary stenosis less than 50% or just with plaques in coronary arteries were considered as atherosclerosis. CAD was further categorized into stable angina, unstable angina and myocardial infarction (MI) according to clinical manifestation and electrocardiogram. Unstable angina, ST-elevation MI and non-ST-elevation MI are collectively referred to as acute coronary syndrome (ACS). Gensini score and number of diseased coronary branches were employed to assess the severity of CAD (Song et al. 2022). To calculate Gensini score, points were assigned based on the size, importance, and location of stenosis in the coronary artery tree, and weighted processing was performed. To calculate the number of diseased coronary branches, diseased branches were assigned based on the importance of the coronary arteries, i.e., 2 diseased branches for LM stenosis, and 1 diseased branch for LAD, LCX and RCA. Finally, the total number of diseased branches was obtained by weighted processing.

Statistical analysis

Statistical analysis was conducted by using 15.0 version SPSS (SPSS, Inc., Chicago, Illinois, USA). Continuous data were expressed as mean ± standard error (SE). Data were tested for normality, and log transformation was performed for statistical analysis if they did not conform to a normal distribution. Differences among the subjects with normal coronary arteries, atherosclerosis and CAD were analyzed by using one-way ANOVA analysis for continuous variables, and Chi-square test for categorical variables. Pearson correlation analysis was conducted to analyze the correlations between circNIPSNAP3A or circHIPK3 and cardiovascular risk factors in the entire study population. Differences among the percentiles of circNIPSNAP3A and circHIPK3 were analyzed by using one-way ANOVA analysis for continuous variables, and Chi-square test for categorical variables. Univariate and multivariate logistic regression analyses were performed to analyze the correlation between circNIPSNAP3A and atherosclerosis or CAD. All P values were two-tailed and the differences were considered as significant if $P \leq 0.05$.

Results

Clinical characteristics of the study population

Clinical characteristics of the study population are shown in Table 1. Both CAD and atherosclerosis groups had older age, higher prevalence of hypertension, and higher expression of circNIPSNAP3A than the normal coronary artery group ($P < 0.05$ for all). The CAD group had a higher prevalence of male gender, smoking and T2DM, higher levels of UA, glucose, CysC and homocysteine, and lower levels of HDL-C and APOAI than the normal coronary artery group or the atherosclerosis group or both ($P < 0.05$ for all). The atherosclerosis group had a higher expression of circHIPK3 than the normal coronary artery group or the CAD group ($P < 0.05$ for both).

Cardiovascular risk factor profiles by percentiles of circNIPSNAP3A

The results of Pearson correlation analyses showed that circNIPSNAP3A is positively correlated with triglycerides, UA and homocysteine, but negatively correlated with HDL-C and APOAI ($P < 0.05$ for all) (Table 2). The major cardiovascular risk factors of the subjects with different percentiles of circNIPSNAP3A are shown in Table 3. The subjects with a high percentage (> 66th percentile) of circNIPSNAP3A had a higher mean level of triglycerides than those with a low percentage (< 33rd percentile) in the normal coronary artery group ($P < 0.05$). The subjects with a high percentage of circNIPSNAP3A had lower average levels of HDL-C and APOAI than those with a low or medium percentage (33rd-66th percentile) of circNIPSNAP3A in the atherosclerosis group ($P < 0.05$ for all). The subjects with a medium or high percentage of circNIPSNAP3A had a higher mean level of UA than those below 33rd percentile in the atherosclerosis group ($P < 0.05$ for both). The subjects with a high percentage of circNIPSNAP3A had higher mean levels of triglycerides and homocysteine than those with a low percentage of circNIPSNAP3A in the CAD group ($P < 0.05$ for both). No correlation was found between circNIPSNAP3A and TC, LDL-C, APOB, Lp(a) or glucose in the present study population. The number of diseased coronary vessels increased orderly with the < 33rd, 33rd-66th, and > 66th percentiles of circNIPSNAP3A in the CAD group, and the patients exceeding > 66th percentile of circNIPSNAP3A had more diseased branches than those with a low percentage of circNIPSNAP3A ($P < 0.05$).

Cardiovascular risk factor profiles by percentiles of circHIPK3

The results of Pearson correlation analyses showed that circHIPK3 is negatively correlated with APOAI ($P <$

Table 1. Clinical characteristics of the study population.

Variables	Normal coronary artery group (n = 83)	Atherosclerosis group (n = 84)	CAD group (n = 171)	*P*
Age, years	60.07 ± 1.43	64.26 ± 1.35[a]	64.99 ± 0.98[a]	0.01
Sex, M/F	35/48	44/40	129/42[a,b]	< 0.001
Weight, kg	63.92 ± 1.38	64.44 ± 1.14	64.58 ± 0.93	0.92
BMI, kg/m^2	24.53 ± 0.49	24.56 ± 0.31	24.38 ± 0.26	0.91
Smoking, n (%)	23 (27.71)	26 (30.95)	87 (50.88)[a,b]	< 0.001
Hypertension, n (%)	33 (39.76)	55 (65.48)[a]	114 (66.67)[a]	< 0.001
T2DM, n (%)	11 (13.25)	20 (23.81)	60 (35.09)[a]	0.001
Triglycerides, mmol/L	1.86 ± 0.23	1.78 ± 0.15	2.02 ± 0.11	0.51
TC, mmol/L	4.61 ± 0.12	4.65 ± 0.12	4.75 ± 0.12	0.71
LDL-C, mmol/L	2.44 ± 0.08	2.52 ± 0.08	2.67 ± 0.08	0.13
HDL-C, mmol/L	1.52 ± 0.04	1.47 ± 0.04	1.34 ± 0.03[a,b]	0.001
APOAI, g/L	1.40 ± 0.04	1.42 ± 0.04[a]	1.26 ± 0.03[a,b]	0.001
APOB, g/L	0.83 ± 0.03	0.85 ± 0.03	0.86 ± 0.03	0.71
Lp(a), mg/L	183.07 ± 23.01	204.66 ± 22.45	212.56 ± 18.89	0.64
UA, μmol/L	331.38 ± 9.70	337.85 ± 10.99	363.86 ± 8.07[a,b]	0.03
Glucose, mmol/L	6.12 ± 0.22	6.17 ± 0.19	7.74 ± 0.28[a,b]	< 0.001
CysC, mg/L	0.85 ± 0.03	0.87 ± 0.03	1.00 ± 0.04[a,b]	0.01
Hs-CRP, mg/L	11.61 ± 2.83	6.56 ± 2.64	13.30 ± 2.35	0.22
Homocysteine, μmol/L	14.78 ± 0.65	14.76 ± 0.58	17.28 ± 0.68[a,b]	< 0.01
CircNIPSNAP3A	0.54 ± 0.04	0.78 ± 0.06[a]	0.81 ± 0.06[a]	< 0.01
CircHIPK3	1.81 ± 0.24	3.43 ± 0.72[a]	2.11 ± 0.24[b]	0.02

CAD, coronary artery disease; M, male; F, female; BMI, body mass index; T2DM, type 2 diabetes mellitus; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; APOAI, apolipoprotein AI; APOB, apolipoprotein B; Lp(a), lipoprotein (a); UA, uric acid; CysC, cystatin C; Hs-CRP, hypersensitive C-reactive protein. [a]$P \leq 0.05$ compared with the normal coronary artery group; [b]$P \leq 0.05$ compared with the atherosclerosis group.

Table 2. Correlation analyses between circNIPSNAP3A or circHIPK3 and cardiovascular risk factors in the entire study population.

Variables	circNIPSNAP3A		circHIPK3	
	r	*P*	r	*P*
Triglycerides	0.44	< 0.001	0.08	0.14
TC	0.04	0.46	0.03	0.65
LDL-C	0.008	0.89	0.002	0.97
HDL-C	−0.18	0.01	−0.10	0.06
APOAI	−0.17	0.02	−0.21	0.001
APOB	0.05	0.44	0.03	0.61
Lp(a)	0.06	0.34	0.07	0.24
Glucose	0.001	0.99	0.07	0.21
UA	0.14	0.03	−0.08	0.16
Homocysteine	0.12	0.04	0.02	0.78

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; APOAI, apolipoprotein AI; APOB, apolipoprotein B; Lp(a), lipoprotein (a); UA, uric acid.

0.05) (Table 2). The major cardiovascular risk factors of the subjects with different percentiles of circHIPK3 are shown in Table 4. The subjects with a medium (33rd-66th percentile) or high percentage (> 66th percentile) of circHIPK3 had a lower mean level of APOAI than those with a low percentage (< 33rd percentile) in the normal coronary artery group ($P < 0.05$ for both). No other significant differences in cardiovascular risk factors including triglycerides, TC, LDL-C, HDL-C, APOAI, APOB, Lp(a), glucose, UA and homocysteine were detected among the subjects with different percentiles of circHIPK3 in the normal coronary artery, atherosclerosis and CAD groups. CircHIPK3 was found not to be related to the severity of CAD when it was evaluated with Gensini score and the number of diseased coronary vessels.

The incidence and severity of CAD in subjects with different percentiles of circNIPSNAP3A and circHIPK3

As compared with the normal coronary artery group, the frequencies of atherosclerosis ($P = 0.01$), stable angina pectoris (SAP) ($P < 0.01$) and unstable angina pectoris (UAP) ($P < 0.001$) increased orderly with the < 33rd, 33rd-66th, and > 66th percentiles of circNIPSNAP3A. In addition, the incidence of ACS was higher than that of atherosclerosis ($P = 0.02$) or SAP ($P = 0.048$) in the patients exceeding > 66th percentile of circNIPSNAP3A (Table 5). Univariate and multivariate logistic regression analyses indicated that circNIPSNAP3A is significantly and independently related to CAD after adjustment for age, sex, hyper-

Table 3. Cardiovascular risk factor profiles by percentiles of circNIPSNAP3A.

Variables	Low circNIPSNAP3A (< 33rd percentile)	Medium circNIPSNAP3A (33rd-66th percentile)	High circNIPSNAP3A (> 66th percentile)	*P*
Normal coronary artery group				
Triglycerides, mmol/L	1.36 ± 0.09	1.91 ± 0.28	2.54 ± 0.68[a]	0.04
TC, mmol/L	4.47 ± 0.16	4.42 ± 0.25	4.97 ± 0.24	0.14
LDL-C, mmol/L	2.33 ± 0.11	2.38 ± 0.18	2.65 ± 1.17	0.15
HDL-C, mmol/L	1.57 ± 0.06	1.56 ± 0.09	1.35 ± 0.09	0.11
APOAI, g/L	1.47 ± 0.08	1.41 ± 0.06	1.29 ± 0.07	0.19
APOB, g/L	0.85 ± 0.05	0.85 ± 0.05	0.76 ± 0.07	0.48
Lp(a), mg/L	144.3 ± 34.1	161.2 ± 35.7	237.6 ± 45.7	0.21
Glucose, mmol/L	6.07 ± 0.59	5.98 ± 0.43	6.24 ± 0.27	0.88
UA, μmol/L	327.4 ± 15.5	320.2 ± 15.1	353.2 ± 20.5	0.43
Homocysteine, μmol/L	14.59 ± 1.31	14.15 ± 1.04	15.81 ± 0.94	0.59
Atherosclerosis group				
Triglycerides, mmol/L	1.28 ± 0.13	1.89 ± 0.29	1.94 ± 0.24	0.20
TC, mmol/L	4.43 ± 0.18	4.58 ± 0.28	4.92 ± 0.19	0.18
LDL-C, mmol/L	2.43 ± 0.20	2.43 ± 0.13	2.67 ± 0.13	0.37
HDL-C, mmol/L	1.58 ± 0.08	1.53 ± 0.06	1.34 ± 0.06[a,b]	0.05
APOAI, g/L	1.52 ± 0.11	1.50 ± 0.07	1.29 ± 0.05[a,b]	0.03
APOB, g/L	0.79 ± 0.07	0.82 ± 0.05	0.93 ± 0.05	0.19
Lp(a), mg/L	119.5 ± 25.4	217.9 ± 30.9	239.8 ± 44.0	0.11
Glucose, mmol/L	5.79 ± 0.23	6.06 ± 0.27	6.46 ± 0.37	0.38
UA, μmol/L	279.4 ± 17.8	347.7 ± 16.9[a]	361.8 ± 18.7[a]	0.01
Homocysteine, μmol/L	14.26 ± 1.28	14.85 ± 1.01	14.95 ± 0.81	0.90
CAD group				
Triglycerides, mmol/L	1.75 ± 0.12	1.97 ± 1.17	2.37 ± 0.27[a]	0.05
TC, mmol/L	4.77 ± 0.20	4.82 ± 0.28	4.68 ± 0.15	0.89
LDL-C, mmol/L	2.65 ± 0.13	2.73 ± 0.18	2.63 ± 0.10	0.78
HDL-C, mmol/L	1.36 ± 0.06	1.33 ± 0.04	1.32 ± 0.04	0.85
APOAI, g/L	1.31 ± 0.05	1.26 ± 0.04	1.23 ± 0.05	0.53
APOB, g/L	0.87 ± 0.05	0.85 ± 0.04	0.87 ± 0.05	0.90
Lp(a), mg/L	174.9 ± 31.6	207.8 ± 30.9	246.2 ± 34.3	0.31
Glucose, mmol/L	7.32 ± 0.44	7.60 ± 0.47	8.29 ± 0.55	0.37
UA, μmol/L	353.5 ± 12.2	347.4 ± 13.8	391.6 ± 15.6	0.66
Homocysteine, μmol/L	14.86 ± 0.68	18.03 ± 1.32	18.52 ± 1.21[a]	0.03
Gensini scores	29.30 ± 2.64	31.55 ± 3.32	35.71 ± 3.95	0.38
Diseased branches	1.82 ± 0.09	2.13 ± 0.11	2.30 ± 0.14[a]	0.01

CAD, coronary artery disease; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; APOAI, apolipoprotein AI; APOB, apolipoprotein B; Lp(a), lipoprotein (a); UA, uric acid. [a]$P \leq 0.05$ compared with the low circNIPSNAP3A group; [b]$P \leq 0.05$ compared with the medium circNIPSNAP3A group.

tension, diabetes, smoking, triglycerides, TC, LDL-C, HDL-C, APOAI, APOB, Lp(a), glucose, UA and homocysteine (Table 6). No significant differences in the incidence and severity of CAD among the < 33rd, 33rd-66th, and > 66th percentiles of circHIPK3 were found in this study.

Discussion

The present study demonstrates that serum circNIPSNAP3A is significantly associated with cardiovasucar risk factors such as triglycerides, HDL-C, APOAI, UA and homocysteine, which in turn confers a higher risk of atherosclerosis and CAD. To the best of our knowledge, this is the first time to report a significant association of serum circNIPSNAP3A with occurrence and development of CAD, although circNIPSNAP3A was previously reported to be up-regulated in the peripheral blood mononuclear cells and atherosclerotic plaques of patients with CAD (Wang et al. 2019; Li et al. 2021). Regarding serum circHIPK3, a significant association with APOAI was detected in the subjects with normal coronary arteries, but there was no significant association with atherosclerosis, CAD or severity of CAD being found.

Table 4. Cardiovascular risk factor profiles by percentiles of circHIPK3.

Variables	Low circHIPK3 (< 33rd percentile)	Medium circHIPK3 (33rd-66th percentile)	High circHIPK3 (> 66th percentile)	*P*
Normal coronary artery group				
Triglycerides, mmol/L	1.30 ± 0.10	1.77 ± 0.21	2.42 ± 0.63	0.17
TC, mmol/L	4.61 ± 0.22	4.56 ± 0.14	4.68 ± 0.27	0.91
LDL-C, mmol/L	2.41 ± 0.16	2.41 ± 0.11	2.50 ± 0.18	0.88
HDL-C, mmol/L	1.63 ± 0.08	1.49 ± 0.06	1.45 ± 0.08	0.25
APOAI, g/L	1.61 ± 0.09	1.31 ± 0.06[a]	1.35 ± 0.05[a]	< 0.01
APOB, g/L	0.80 ± 0.06	0.83 ± 0.05	0.86 ± 0.06	0.79
Lp(a), mg/L	155.4 ± 34.8	184.9 ± 36.7	218.3 ± 52.5	0.58
Glucose, mmol/L	6.22 ± 0.34	5.68 ± 0.31	6.53 ± 0.49	0.26
UA, μmol/L	337.5 ± 16.1	324.2 ± 14.1	333.9 ± 21.9	0.84
Homocysteine, μmol/L	14.06 ± 1.60	13.87 ± 0.70	16.33 ± 1.18	0.21
Atherosclerosis group				
Triglycerides, mmol/L	1.55 ± 0.15	2.12 ± 0.41	1.78 ± 0.22	0.29
TC, mmol/L	4.40 ± 0.25	4.86 ± 0.23	4.67 ± 0.16	0.33
LDL-C, mmol/L	2.34 ± 0.16	2.67 ± 0.17	2.53 ± 0.11	0.32
HDL-C, mmol/L	1.51 ± 0.07	1.47±0.08	1.39 ± 0.07	0.56
APOAI, g/L	1.46 ± 0.07	1.40 ± 0.06	1.41 ± 0.08	0.86
APOB, g/L	0.79 ± 0.04	0.89 ± 0.06	0.89 ± 0.07	0.34
Lp(a), mg/L	171.2 ± 32.6	209.4 ± 36.4	242.5 ± 49.4	0.43
Glucose, mmol/L	6.02 ± 0.32	6.07 ± 0.31	6.49 ± 0.38	0.57
UA, μmol/L	329.2 ± 14.8	351.8 ± 25.7	336.2 ± 18.3	0.69
Homocysteine, μmol/L	14.22 ± 0.78	15.14 ± 1.06	14.86 ± 1.08	0.82
CAD group				
Triglycerides, mmol/L	1.65 ± 0.12	2.30 ± 0.24	2.10 ± 0.19	0.06
TC, mmol/L	4.54 ± 0.16	4.95 ± 0.26	4.77 ± 0.19	0.38
LDL-C, mmol/L	2.53 ± 0.12	2.79 ± 0.15	2.70 ± 0.14	0.40
HDL-C, mmol/L	1.39 ± 0.05	1.28 ± 0.04	1.35 ± 0.05	0.22
APOAI, g/L	1.30 ± 0.05	1.25 ± 0.05	1.24 ± 0.04	0.68
APOB, g/L	0.87 ± 0.04	0.87 ± 0.04	0.85 ± 0.05	0.95
Lp(a), mg/L	235.1 ± 30.4	173.2 ± 29.3	233.3 ± 39.9	0.30
Glucose, mmol/L	7.02 ± 0.40	8.39 ± 0.55	7.86 ± 0.51	0.14
UA, μmol/L	353.7 ± 13.2	372.8 ± 16.1	365.2 ± 12.6	0.63
Homocysteine, μmol/L	16.05 ± 0.74	17.99 ± 1.09	17.99 ± 1.75	0.39
Gensini scores	27.25 ± 3.05	31.90 ± 3.47	37.09 ± 3.34	0.11
Diseased branches	1.88 ± 0.13	2.16 ± 0.12	2.19 ± 0.10	0.12

CAD, coronary artery disease; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; APOAI, apolipoprotein AI; APOB, apolipoprotein B; Lp(a), lipoprotein (a); UA, uric acid. [a]$P \leq 0.05$ compared with the low circNIPSNAP3A group.

CAD is a multifactorial disease with a high morbidity and mortality, causing a huge burden on social economies (Fadah and Payan-Schober 2023; Wein et al. 2023). Discovering and identifying early biomarkers of CAD are crucial for prevention, treatment and prognosis of this severe disease (Abaspour et al. 2019; Li et al. 2020). CircRNAs are more stable than linear RNAs because of their circular structure and resistance to RNA exonucleases, which makes them a good biomarker for human diseases (Meng et al. 2017; Lei et al. 2019). By means of computational analysis, Wang et al. (2019) selected two candidate circRNAs (circNIPSNAP3A and hsa_circ_0004104) to explore their diagnostic value as CAD biomarkers in large cohorts, and found that circNIPSNAP3A is significantly up-regulated in CAD patients with an area under the curve of 0.703. However, previous studies only investigated the expression of circNIPSNAP3A in blood mononuclear cells and atherosclerotic plaques, not in serum. It is well-known that serum is the most important, common, and easily obtainable clinical specimen in clinical testing. In this study, we screened more than 30 circRNAs which were previously reported to be associated with CAD, and found that

Table 5. The incidence and severity of CAD in subjects with different percentiles of circNIPSNAP3A or circHIPK3.

CircRNAs	Low percentage (< 33rd percentile)	Medium percentage (33rd-66th percentile)	High percentage (> 66th percentile)	*P1*	*P2*	*P3*
CircNIPSNAP3A						
Normal coronary artery, n (%)	37 (44.6%)	26 (31.3%)	20 (24.1%)			
Atherosclerosis, n (%)	22 (26.2%)	30 (35.7%)	32 (38.1%)	0.01		
SAP, n (%)	20 (23.3%)	32 (37.2%)	34 (39.5%)	< 0.01	0.72	
ACS, n (%)	10 (11.8%)	32 (37.6%)	43 (50.6%)	< 0.001	0.02	0.048
CircHIPK3						
Healthy coronary artery, n (%)	28 (33.7%)	32 (38.6%)	23 (27.7%)			
Atherosclerosis, n (%)	25 (29.8%)	24 (28.6%)	35 (41.7%)	0.16		
SAP, n (%)	24 (27.9%)	34 (39.5%)	28 (32.6%)	0.38	0.56	
ACS, n (%)	34 (40.0%)	22 (25.9%)	29 (34.1%)	0.99	0.18	0.4

SAP, stable angina pectoris; ACS, acute coronary syndrome. P1, compared with the normal coronary artery group; P2, compared with the atherosclerosis group; P3, compared with the SAP group.

Table 6. Univariate and multivariate logistic regression analyses between circNIPSNAP3A and atherosclerosis or CAD.

Models	Model 1		Model 2		Model 3	
	OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Atherosclerosis	3.22 (1.54-6.71)	< 0.01	2.91 (1.32-6.43)	< 0.01	2.36 (0.91-6.14)	0.08
CAD	2.94 (1.55-5.57)	0.001	2.06 (1.09-3.87)	0.03	2.63 (1.04-6.66)	0.04

Model 1, univariate logistic regression analysis; Model 2, adjusted for age, sex, hypertension, diabetes and smoking; Model 3, adjust for age, sex, hypertension, diabetes, smoking, triglycerides, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, apolipoprotein AI, apolipoprotein B, lipoprotein (a), glucose, uric acid and homocysteine. CAD, coronary artery disease; OR, odds ratio; 95% CI, 95% confidence interval.

circNIPSNAP3A and circHIPK3 have high expression levels in the serum with circNIPSNAP3A being the highest (data not shown). We found that serum circNIPSNAP3A is significantly increased among the atherosclerosis and CAD patients as compared to the subjects with normal coronary arteries. Furthermore, circNIPSNAP3A was shown to be associated with the severity of CAD, since subjects with a high percentage of circNIPSNAP3A had more diseased coronary branches and a higher incidence of ACS. This has a great clinical value in diagnosis, prognosis, and treatment of CAD. Coronary angiography is generally used to evaluate the number of coronary artery lesions and severity of CAD. However, coronary angiography is invasive and costly, and requires advanced operating techniques and equipment. CircNIPSNAP3A can be detected using serum and PCR technology, greatly reducing both trauma and cost. Among high-risk populations for CAD, the detection of circNIPSNAP3A can serve as a preliminary screening method. Once elevated serum levels of circNIPSNAP3A are detected, further coronary angiography can be used to confirm the diagnosis. In addition, if circNIPSNAP3A is the initiating factor of CAD, gene silencing technology can be used to silence it, reduce its serum levels, and achieve the goal of treating CAD.

The mechanisms underlying the associations between circNIPSNAP3A and cardiovascular risk factors are unclear. One possible mechanism is that circNIPSNAP3A promotes the expression of histone deacetylase 9 (HDAC9) by sponging miR-6873-5p (Li et al. 2021). HDAC9 has been reported to be involved in the pathogenesis of dyslipidemia, atherosclerosis and cardiovascular diseases via deacetylation of histones at the promoters of some metabolism-related genes, such as nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ), ATP-binding cassette transporter 1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1), and inhibition of the expressions of these genes (Cao et al. 2014; Das and Natarajan 2020). PPARγ, ABCA1 and ABCG1 are the key genes in regulating glucolipid metabolism, and reduction of PPARγ, ABCA1 and ABCG1 is known to impair lipolysis and slow cholesterol reverse transport, which in turn increases triglyceride, UA and homocysteine levels and decreases HDL-C and APOAI levels (Li et al. 2022; Song et al. 2022, 2023b; Said et al. 2023). Hypertriglyceridemia, hypercholesterolemia, hypo-high-density lipoproteinemia, hyperuricemia and homocystinemia are well-known to be the main risk factors for CAD. Therefore, the elevated circNIPSNAP3A level in serum promotes the occurrence and progression of atherosclerosis and CAD. CircNIPSNAP3A may also work as a protein ligand. Some circRNAs function through direct binding to target proteins and regulating their activities. CircUSP9X was found to be up-regulated

in atherosclerosis and ox-LDL-treated HUVECs, and mechanistically, circUSP9X directly binds to and inhibits GSDMD, a key protein in the pyroptosis pathway (Wang and Ruan 2023, Xu et al. 2023). As a result, endothelial pyroptosis is inhibited, and atherosclerosis occurs and develops. Several other mechanisms, such as working as a template for translation and interaction with DNA or RNA, may also be involved in the working principle of circNIPSNAP3A on CAD.

The study did not find significant differences in the incidence and severity of CAD across percentiles of circHIPK3 although a significant relationship between circHIPK3 and APOAI levels was noted. CAD is a multifactorial disease, and there are many risk factors involved in the occurrence and progression of CAD, such as hyperlipidemia, hyperuricemia, hyperhomocysteinemia, smoking, and old age (Zhang et al. 2019; Walli-Attaei et al. 2020). The higher the level and number of risk factors, the greater the risk of CAD. In this study, we found that circHIPK3 was only significantly negatively correlated with APOAI. Subjects with higher levels of circHIPK3 had lower APOAI levels, and no associations were detected between circHIPK3 and other cardiovascular risk factors. Therefore, circHIPK3 is just weakly correlated with cardiovascular risk factors and has a low risk of cardiovascular diseases. In addition, circHIPK3 was detected to be significantly elevated in the sera of atherosclerosis patients compared to the normal coronary artery group, but somehow to be significantly decreased in the sera of CAD patients as compared with the normal coronary group. Several previous studies have also examined the relationship and underlying mechanisms between circHIPK3 and atherosclerosis (Wei et al. 2020; Kang et al. 2021; Zhang et al. 2022). Zhang et al. (2022) found that the expression of circHIPK3 was down-regulated in the tissues and blood samples of patients with atherosclerosis, and circHIPK3 inhibited the calcification of vascular smooth muscle cells via miR-106a-5p/MFN2 axis. Wei et al. (2020) observed that the expression of circHIPK3 was down-regulated in mice with a high-fat diet and ox-LDL-treated HUVECs, and circHIPK3 protected endothelial cells from dysfunction by promoting autophagy, while Kang et al. (2021) demonstrated that circHIPK3 promoted the apoptosis of VSMCs by influencing the miR-637/CDK6 axis. The mechanisms of circHIPK3 on the occurrence and development of atherosclerosis are somewhat confusing, and more researches are needed to elucidate the mechanism of action underlying the anti-atherosclerotic effect of circHIPK3.

The present study has limitations. Firstly, the depth of mechanism exploration is not sufficient. We just examined the relation of the two circRNAs of circNIPSNAP3A and circHIPK3 with CAD risk factors as well as CAD severity, and did not investigate the mechanisms underlying the associations of circNIPSNAP3A with atherogenic metabolic abnormalities, atherosclerosis and CAD severity. Thus, we will further do *in vitro* and *in vivo* researches to explore the mechanism of action of circNIPSNAP3A on the etiology of CAD in the follow-up researches. Secondly, the subjects enrolled in this study were exclusively Chinese Han people living in Chengdu city, Southwest China, and therefore the findings of the present study may not apply to other ethnic origins.

In conclusion, serum circNIPSNAP3A is associated with atherogenic metabolic abnormalities, atherosclerosis and severity of CAD, while circHIPK3 has a weak correlation with dyslipidemia.

Author Contributions

Song, Y.Y. and Wang, X. conceived of the study, participated in the design, analyzed the data, and drafted the manuscript. Nie, H.Y., Su, M., Wu, Y., Pang, Q.Y., Zhang, Y.J. and He, C. carried out sample collection, conducted molecular experiments, and revised the manuscript critically for important intellectual content. All authors reviewed and approved the final version of the manuscript for submission.

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Conflict of Interest

The authors declare no conflict of interest.

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