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Original Article

Association of circular RNA hsa_circ_0124644 and incidence and severity of coronary heart disease in premenopausal women

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Abstract: Background: Coronary heart disease (CHD) is a prevalent cause of mortality, and its incidence has exhibited an upward trajectory in recent times, particularly among younger individuals. Circular RNAs (circRNAs) have emerged as promising biomarkers for early detection in various cancer types. The objective of this study was to investigate the correlation between circ_0124644 and coronary heart disease (CHD) in premenopausal women. Methods: A total of 60 premenopausal women were selected for the CHD group, while another 60 were chosen for the non-CHD group, with the mean age of 50.89 ± 3.79 years. Subsequently, Pearson analysis was employed to examine the correlation between various indicators. Quantitative polymerase chain reaction (qPCR) was employed for the detection of hsa_circ_0124644 expression in tissues obtained from patients. The receiver operating characteristic (ROC) curve's area under the curve (AUC) was utilized to establish the diagnostic cut-offs. To examine the impact of hsa_circ_0124644 on CHD, multivariate logistic regression and multiplicative analysis were conducted. Results: The findings from the Pearson analysis showed a significant correlation between the expression of hsa-circ-0124644 and the severity of CHD in premenopausal women ($P<0.001$, $r=0.943^{**}$). Further validation through qPCR demonstrated that the levels of hsa_circ_0124644 expression in peripheral blood leukocytes were significantly elevated in premenopausal women with CHD compared to those without CHD (all $P<0.05$). Moreover, there was a statistically significant association between the expression of hsa-circ-0124644 and a higher occurrence of CHD in premenopausal females ($P<0.001$). Additionally, the AUC of ROC curve was found to be 0.753, with a threshold of hsa-circ-0124644 expression at 0.509, resulting in a sensitivity of 100% and specificity of 50.9%. Conclusions: The findings of this study indicate a plausible correlation between hsa-circ-0124644 and the etiology and advancement of CHD in premenopausal females, thereby underscoring its prospective application as a biomarker for CHD.

Keywords: Coronary heart disease, circular RNA, hsa_circ_0124644, menopause

Introduction

Coronary heart disease (CHD) is prevalent among women and is characterized by the constriction of the coronary arteries, leading to severe health consequences [1, 2]. It is widely acknowledged that the decline in estrogen levels, a hormone known for its atherosclerosis-preventing properties [3], significantly contributes to the heightened occurrence of CHD in women following menopause [4, 5]. Hence, recent scholarly investigations have primarily concentrated on CHD among postmenopausal women. However, there is an observable inclination towards the occurrence of CHD at younger ages, with reports indicating that premenopausal women experience atypical symptoms but exhibit a faster and more urgent onset of the disease compared to their postmenopausal counterparts [6]. Presently, there is a dearth of

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effective early diagnostic approaches specifically tailored for identifying CHD in premenopausal women.

The primary characteristic of circular RNAs (circRNAs) is their formation of an annular structure by joining the 5' and 3' terminals. These structures have the potential to exert substantial influence on gene expression regulation, serving as scaffolds for RNA or proteins, microRNA (miRNA) sponges, and splicing regulators [7-10]. Therefore, by virtue of these attributes, circRNAs have the potential to function as innovative biomarkers in various cancer types, facilitating precise differentiation and discernment of tumor tissues from adjacent healthy tissues [11-13]. According to the results obtained from these studies, it is postulated that circRNA could serve as a promising biomarker for CHD in premenopausal women. Nevertheless, it is worth noting that no prior research has investigated the feasibility of utilizing circRNAs for diagnosing CHD in this specific population.

The objective of this retrospective study, comprising 120 patients, was twofold: (1) to explore the potential disparity in RNA hsa_circ_0124644 levels between premenopausal women with and without CHD, and (2) to assess the association between the severity of CHD and the extent of RNA hsa_circ_0124644 expression in premenopausal women.

Materials and methods

Study population

The CHD group consisted of 60 women diagnosed with CHD who were admitted to our hospital between January 2021 and June 2022, while the control group comprised 60 healthy women. The inclusion criteria encompassed the following [14]: ① Patients diagnosed with CHD for the first time and CHD included: (i) The presence of significant stenosis (≥ 50%) in more than one major coronary artery was confirmed through cardiac catheterization. (ii) A documented history of previous myocardial infarction (MI) was observed. (iii) The study includes patients in the stable stage after acute MI and patients exhibiting ST-segment elevation or depression on electrocardiogram (ECG). ② The study population was restricted to individuals aged between 40 and 55 years. The exclusion criteria encompassed the following: ① Patients who used hormones or hormone-related pharmaceuticals for a duration of three months were excluded; ② Individuals who had experienced a stroke, significant injury, or undergone major surgery within a six-month period, or had a previous history of cancer or a newly developed tumor. Additionally, pregnant or breastfeeding women were excluded; ③ Other forms of cardiac disease, severe organic brain disorders, impaired liver or kidney function, recent infections, and endocrine system disorders were also considered exclusionary factors; ④ Patients who were no longer available for follow-up were also excluded.

Data collection

The data collected for each patient encompassed sociodemographic information, such as age, sex, BMI, drinking and smoking habits, as well as clinical information, including medical history, HbA1c, homocysteine, luteinizing hormone, oestradiol, progesterone, follicle-stimulating hormone (FSH), prolactin (PRL), creatinine (Cre), low-density lipoproteins (LDL), triglycerides (TG), ejection fraction (EF), end-systolic dimension (LVS), right ventricular (RV) diameter, left ventricular end-diastolic diameter (LVD), left atrial (LA) diameter, and Gensini score [15].

Blood samples and RNA extraction

A 5 ml blood sample was collected from the antecubital vein of each participant and transferred into commercially available plasma tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). Following this, the blood samples were stored at a temperature of -80°C. Total RNA was extracted from 1 ml of peripheral blood collected within 20 minutes using RNA extraction kit (Bioteke, Beijing) in a cohort of 60 patients with CHD and 60 non-CHD controls. After assessing the integrity of the RNA, the reverse transcription process for quantified RNA was executed using a PrimeScript RT reagent kit (Takara Bio, Japan) in accordance with the manufacturer's guidelines. Specifically, a mixture containing 500 ng RNA, 2 μl PrimeScript RT Master Mix (Takara Bio, Japan), and RNase-Free water was prepared, resulting in a final volume of 10 μl. Subsequently, the mixture underwent incubation in a water bath set at 37°C for a duration of 15 minutes,

Table 1. Baseline characteristics of study subjects

Characteristics	Total (N=120)	CHD (N=60)	Non-CHD (N=60)	*p* value	t/X^2
Age (years)	50.89 ± 3.79	51.00 ± 3.66	50.78 ± 3.95	0.421	0.312
BMI (kg/m^2)	25.77 ± 4.07	26.56 ± 4.30	24.99 ± 3.70	0.209	2.148
Smoking				0.309	0.259
Yes	4	3	1		
No	116	57	59		
Alcohol drinking				0.648	0.209
Yes	5	2	3		
No	115	58	57		
Marital status					
Marriage	108	53	55	0.543	0.093
Single and others	12	7	5		

facilitating the completion of reverse transcription and the attainment of total cDNA. For quantitative PCR analysis, a quantity of 2 μl of synthetic cDNA was employed, utilizing the LightCycler 480 PCR System. The reaction system was composed of a volume of 20 μL. The reaction conditions encompassed an initial predenaturation step at 95°C for 30 seconds, followed by PCR amplification at 95°C for 5 seconds and 60°C for 34 seconds, repeated for a total of 45 cycles.

Statistical analysis

The means ± standard deviations were used to report continuous variable data, whereas percentages were employed to present categorical variable data. Statistical analysis involved independent sample t tests for continuous data and chi-squared tests for categorical data. The Gensini score was chosen to assess the severity of CHD and examine the association between the severity of CHD in middle-aged premenopausal women. Pearson's analysis was employed to analyze correlations. The multivariate analysis incorporated univariate analysis of significant variables. The ROC curve was utilized to determine the diagnostic cut-offs of circRNAs. Statistical significance was defined as $P<0.05$. The data analysis was conducted utilizing SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

Results

Baseline demographic characteristics

A total of 120 female patients, with a mean age of 50.89 ± 3.79 years, were enrolled in the study. The distribution of participants based on demographic variables can be found in **Table 1**. No significant difference was detected in preoperative demographic information between the groups (**Table 1**, $P>0.05$).

Univariate logistic regression analysis

All laboratory findings, including HbA1c, homocysteine, LH, oestradiol, progesterone, FSH, PRL, Cre, LDL, TG, EF, LV (d), LV (s), RV, LA, and expression of hsa-circ-0124644, were subjected to analysis using univariate logistic regression. The findings are presented in **Table 2**, wherein HbA1c ($P=0.011$), TG ($P=0.008$), and expression of hsa-circ-0124644 ($P<0.001$) exhibited statistical significance in the univariate analysis.

Multivariate logistic regression analysis

The multivariate logistic regression analysis included age and the three variables that were found to be statistically significant in the univariate analysis. The findings revealed that only the expression of hsa-circ-0124644 was significantly linked to a greater occurrence of coronary heart disease in premenopausal women, as indicated in **Table 3**.

Pearson's analysis

According to the univariate analysis, the relationship between HbA1c, TG, expression of hsa-circ-0124644 and severity of CDH in middle-aged premenopausal women was assessed. The results showed that the Pearson correlation was significant for the expression of hsa-circ-0124644 ($P<0.001$, $r=0.943^{**}$, **Figure 1**)

Table 2. Comparison of laboratory findings and circRNA expressions between the groups

Characteristics	Non-CHD patients (n=60)	CHD patients (n=60)	*p* value	t
HbA1c	6.10 ± 0.50	7.69 ± 1.68	0.011	-2.588
Homocysteine	12.08 ± 3.38	11.25 ± 2.81	0.146	1.465
LH	22.62 ± 12.26	20.26 ± 11.65	0.282	1.081
Estradiol	46.91 ± 31.55	38.70 ± 20.92	0.095	-1.682
Progesterone	2.38 ± 1.70	2.03 ± 1.16	0.194	1.306
FSH	37.11 ± 21.58	35.04 ± 22.28	0.605	0.519
PRL	9.95 ± 5.34	9.82 ± 4.01	0.875	0.157
Cre	67.48 ± 13.56	68.51 ± 13.99	0.684	-0.408
LDL	2.73 ± 0.81	2.68 ± 1.21	0.819	0.229
TG	1.45 ± 0.58	1.83 ± 0.93	0.008	-2.695
EF	66.1 ± 5.35	64.35 ± 9.76	0.226	1.218
LV (d)	45.2 ± 4.24	45.58 ± 4.10	0.616	-0.503
LV (s)	28.78 ± 5.22	29.75 ± 5.94	0.346	-0.947
RV	20.53 ± 4.48	19.58 ± 2.48	0.107	1.623
LA	31.31 ± 5.57	32.75 ± 3.73	0.098	-1.667
Hsa-circ-0124644	1.61 ± 0.63	3.88 ± 0.93	<0.001	-5.543

Table 3. Multivariate logistic regression analysis of independent risk factors

Covariates	Bold values	p	OR	95% CI
Age	-0.093	0.400	0.911	0.734, 1.131
HbA1c	-0.751	0.393	0.472	0.084, 2.642
TG	-0.757	0.245	0.469	0.131, 1.679
Hsa-circ-0124644	-4.190	<0.001	0.015	0.003, 0.090

but not significant for HbA1c (P=0.090, r= 0.221, **Figure 2**) or TG (P=0.605, r=0.068, **Figure 3**).

ROC analysis

The analysis of the ROC curve revealed that the hsa-circ-0124644 expression had an optimal cut-off point of 0.509, with a sensitivity of 100% and a specificity of 50.9%. The AUC was determined to be 0.753, as depicted in **Figure 4**.

Discussion

Perimenopausal women are at an elevated risk of CHD due to the absence of oestrogen's protective effects [16-18]. Existing scholarly literature has identified several risk factors associated with CHD in women, such as family history [19, 20], age [17, 18], smoking [21, 22], dyslipidaemia [23, 24], diabetes [25, 26], hypertension [27], and psychological factors [28, 29]. Nevertheless, it is imperative to acknowledge that women exhibit notable variations in both physical and psychological aspects prior to and following menopause. It is worth noting that the aforementioned studies solely examined the correlation between women and CHD. Conversely, there exists a dearth of research focusing on the risk factors associated with CHD in premenopausal women.

CircRNA, a noncoding RNA, is ubiquitously present in eukaryotes. Its expression remains stable even when subjected to various factors such as RNA enzyme activity, boiling, repeated freeze-thaw cycles, or long-term storage [30]. Furthermore, the expression of circRNA exhibits tissue-specific and disease-specific patterns in the majority of cancerous tissues and plasma samples [31, 32]. The aforementioned characteristics render circRNA a promising biomarker for various diseases, particularly for the timely detection and prognostication of cancer. Within the scope of this investigation, the expression levels of hsa-circ-0124644 were observed to be 1.61 ± 0.63 in non-CHD patients and 3.88 ± 0.93 in CHD patients (P<0.001), aligning with the findings reported in a prior publication [14]. A separate investigation demonstrated that the upregulation of circRNA in cardiac myocyte cells induces cellular apoptosis [33]. Additionally, the prognostic significance of hsa-circ-0124644 expression was assessed. The findings substantiated an AUC of 0.753, accompanied by a sensitivity of 100% and specificity of 50.9%. These outcomes suggest potential for the early anticipation and detection of CHD advancement in middle-aged premenopausal females. In the multivariate analysis, a range of covariates were incorporated to examine the risk factors. The findings substantiated a significant association between hsa-circ-0124644 and an elevated occurrence of CHD in middle-aged premenopausal women.

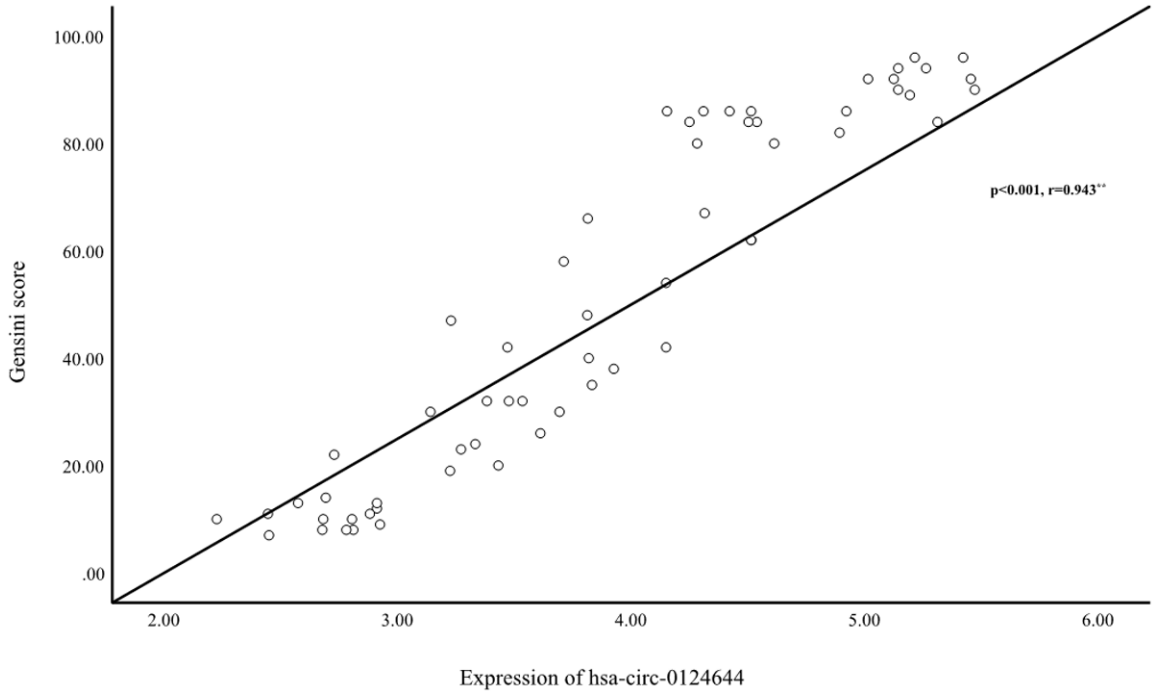

Figure 1. Pearson's analysis of expression of hsa-circ-0124644 and severity of CDH in middle-aged premenopausal women. The results showed that the Pearson correlation was significant for the expression of hsa-circ-0124644 (P<0.001, r=0.943**).

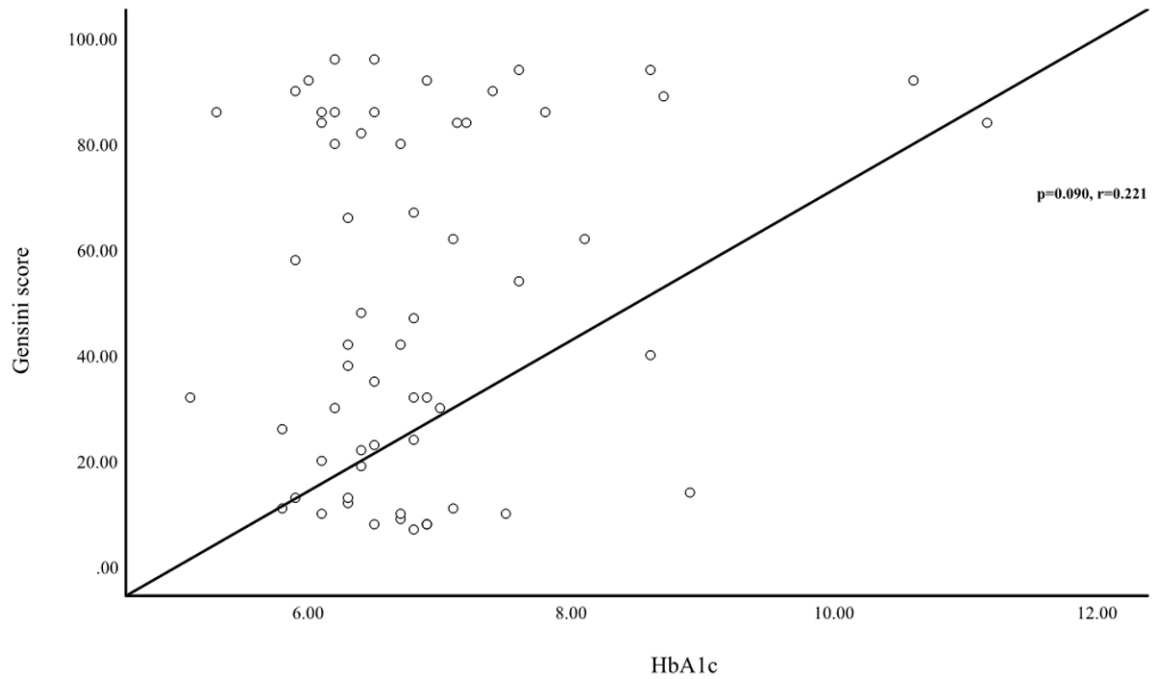

Figure 2. Pearson's analysis of HbA1c and severity of CDH in middle-aged premenopausal women. The results showed that there was no significant correlation between HbA1c and severity of CDH (P=0.090, r=0.221).

These results deviated from those reported in prior studies [19-27]. The primary rationale behind this disparity could be the exclusion of older postmenopausal women from the present study. Consequently, covariates including age, BMI, adverse lifestyle habits, and labora-

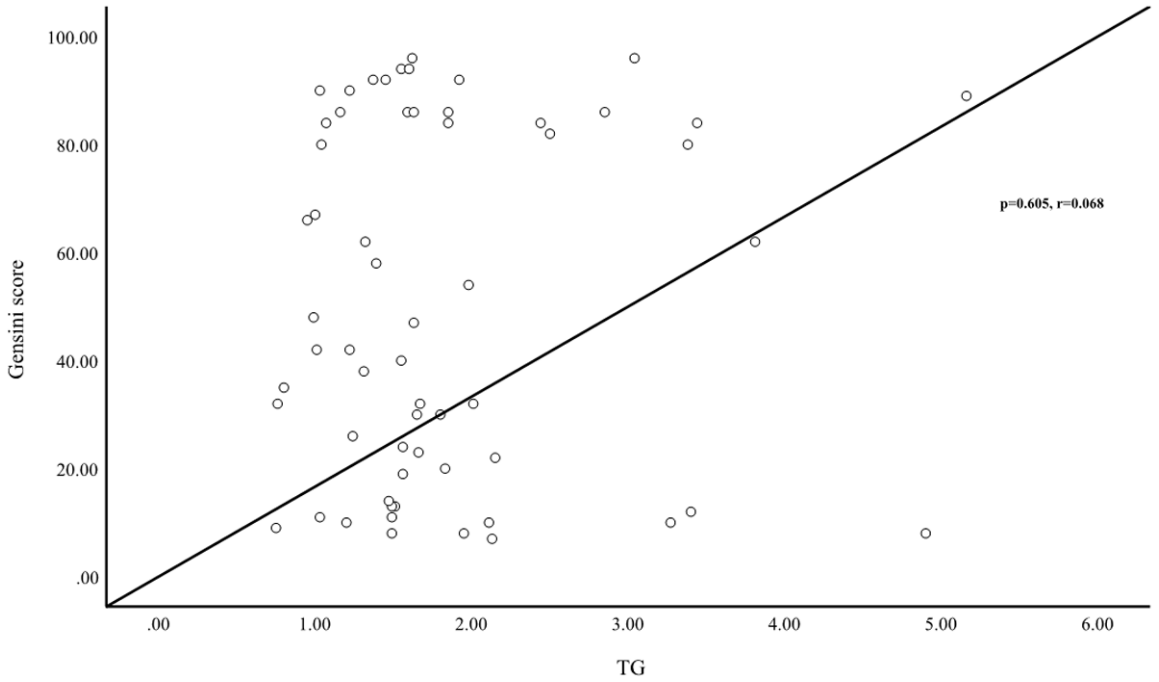

Figure 3. Pearson's analysis of TG and severity of CDH in middle-aged premenopausal women. The results showed that there was no significant correlation between TG and severity of CDH (P=0.605, r=0.068).

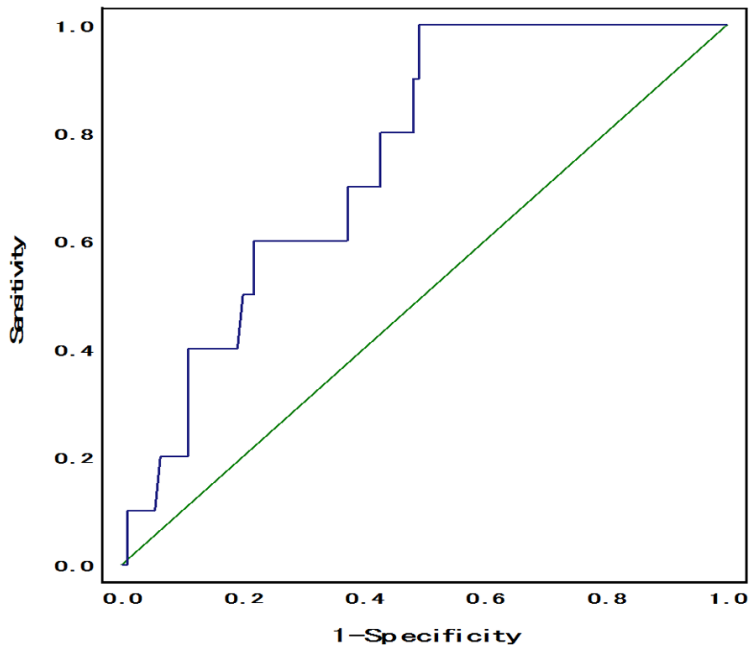

Figure 4. ROC curve for the sensitivity and specificity of expression of hsa-circ-0124644 in predicting CDH in middle-aged premenopausal women. The AUC is 0.753 (sensitivity =100%, specificity =50.9%).

tory outcomes did not exhibit noteworthy distinctions between the two groups.

There exists a correlation between estrogen and circRNAs, primarily attributed to the function of circRNAs as molecular sponges that impact the reduction of estrogen levels. Additionally, when estrogen receptors are moderately affected, the expression of associated circRNAs undergoes alteration, potentially implicating their involvement in the development of coronary heart disease. According to certain scholars, circRNAs are considered a type of endogenous competing RNAs that can function as miRNA sponges. They can also bind to relevant functional proteins, thereby contributing to the activation of inflammatory pathways and the regulation of inflammatory factor expression [34]. The expression levels of circRNAs in certain tissues have been found to be 10 times greater than the expression levels of linear RNAs, thereby establishing cir-

cRNA as a superior biomarker [35]. Specifically, hsa_circ_0124644, a type of circRNA, has been demonstrated to possess significant diagnostic value as a biomarker for CHD [36]. The field of circRNAs is relatively nascent, and thus far, no definitive evidence has been discovered regarding the function of hsa_circ_0124644. Nevertheless, genetic ontological enrichment analysis has indicated a significant association between the hsa_circ_0124644 gene and various cellular processes, including apoptosis and the Robo receptor signalling pathway [37].

The present study is subject to certain limitations that should be acknowledged. Firstly, due to its retrospective nature, patient selection bias was inevitable. Furthermore, the sample size was relatively small, which can be attributed to the relatively lower incidence of CHD in premenopausal women compared to postmenopausal women. Consequently, it is imperative to conduct randomized controlled trials with substantial sample sizes in order to corroborate and validate our findings. Moreover, the absence of prior research investigating the correlation between CIRC-0124644 and CHD in premenopausal women hinders our ability to comprehensively discuss and contrast our findings. Furthermore, the multivariate analysis did not incorporate measures of psychological well-being and quality of life. Incorporating all these risk factors into the logistic regression analysis within a singular study poses a challenge. The primary objective of this study was to examine the correlation between hsa-circ-0124644 and CHD in premenopausal women.

Conclusions

The findings of this study indicate a plausible correlation between hsa-circ-0124644 and the etiology and advancement of CHD in premenopausal females, thereby underscoring its prospective application as a biomarker for CHD.

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Written informed consent were obtained from all the participants.

Disclosure of conflict of interest

None.

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