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Association of S100B 3'UTR polymorphism with risk of chronic heart failure in a Chinese Han population

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Abstract

To study the correlation between single nucleotide polymorphism (SNP) of the 3′ untranslated region (UTR) rs9722 locus in *S100B* and the risk of chronic heart failure (CHF), plasma levels of S100B protein as well as has-miR-340-3p in a Chinese Han population.

A total of 215 patients with CHF (124 ischemic cardiomyopathy (ICM) and 91 dilated cardiomyopathy (DCM)) and 215 healthy controls were recruited to analyze the *S100B* rs9722 genotype by Sanger sequencing. The levels of hsa-miR-340-3p in the plasma were detected by RT-PCR, and S100B levels were detected by ELISA.

The risk of CHF in *S100B* rs9722 locus T allele carriers was 4.24 times higher than that in those with the C allele (95% CI: 2.84–6.33, $P < .001$). The association of *S100B* rs9722 locus SNP with ICM and DCM risk was not affected by factors such as age, gender, and body mass index (BMI). The levels of plasma S100B and hsa-miR-340-3p in patients with ICM and DCM were significantly higher than those in the control group ($P < .001$). There was no significant difference in plasma S100B levels between patients with ICM and DCM ($P > .05$). Among ICM, DCM, and control subjects, TT genotype carriers had the highest levels of plasma S100B and hsa-miR-340-3p, followed by the CT genotype and TT genotype, and the difference was statistically significant ($P < .05$). Plasma hsa-miR-340-3p levels were positively correlated with S100B levels in the control subjects and patients with ICM and DCM.

The *S100B* rs9722 locus SNP is associated with CHF risk in a Chinese Han population.

Abbreviations: BMI = body mass index, CHF = chronic heart failure, DCM = dilated cardiomyopathy, ELISA = enzyme-linked immunosorbent assay, ICM = ischemic cardiomyopathy, LAD = left atrial diameter, LVEDD = left ventricular end-diastolic diameter, LVEDV = left ventricular end-diastolic volume, LVEF = left ventricular ejection fraction, LVESD = left ventricular end-systolic diameter, LVESV = left ventricle end-systolic volume, SNP = single nucleotide polymorphism, UTR = untranslated region.

Keywords: chronic heart failure, microRNA, S100B, single nucleotide polymorphism

1. Introduction

Cardiovascular disease is one of the leading threats to human health. Heart failure is the main end stage in the development of heart disease, which seriously affects the quality of life of patients.[1] Recently, because of its high incidence and mortality rate, chronic heart failure (CHF) has become a research hotspot.[2,3] Previous studies have shown that obesity, renal insufficiency, arteriovenous fistula and amyloid deposition are risk factors for CHF.[4] Besides, several inflammatory factors have been shown to be associated with the onset and prognosis of CHF, such as S100B[5] and hsCRP.[6]

S100B belongs to the S100 protein superfamily, a multi-gene family consisting of EF-hand (helix E-loop-helix F) calcium-binding protein, which has broad biological functions, and plays an important role in cell proliferation, differentiation, promoting gene expression, and cellular regulation.[7–9] It was reported that S100B levels were increased in the pathological state of cardiovascular, pulmonary, and renal diseases.[10] A recent study demonstrated that S100B levels are elevated in patients with coronary heart disease and chronic heart failure, suggesting that S100B levels are associated with the severity of myocardial ischemia, predicting the extent of myocardial infarction, and the severity of cardiac dysfunction in patients with CHF.[11]

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S100B is located at 21q.22.3. According to previous studies, *S100B* rs9722 locus single nucleotide polymorphism (SNP) is associated with the serum or brain S100B levels.[12,13] In the present study, we designed a case-control study to analyze the association between *S100B* rs9722 locus SNP and the risk of CHF in a Chinese Han population.

2. Materials and methods

2.1. Study participants

The study participants comprised 215 Chinese Han patients with CHF, who were recruited from the Second Affiliated Hospital of Hainan Medical University from October 2015 to December 2018, including 124 cases of ICM and 91 cases of DCM. All patients with CHF, ICM and DCM were diagnosed in accordance with the 2005 ACC/AHA Guidelines for the Diagnosis and Management of Adult Heart Failure,[14] with the symptoms and signs of CHF, left ventricular ejection fraction (LVEF) ≤45% confirmed by echocardiographic diagnosis, and cardiac function classified as stage II–III by the New York Heart Association (NYHA). The exclusion criteria were:

(1) acute myocardial infarction or experience of acute myocardial infarction within 3 months;
(2) patients with valvular heart disease without surgical correction;
(3) patients with immune system diseases;
(4) patients with diabetes;
(5) patients with mental disorders; and
(6) pregnant women.

Another cohort of 215 healthy subjects without cardiovascular disease were also recruited, confirmed by echocardiography to have LVEF >45%, without clinical dizziness, chest pain, palpitations, and other discomfort, and with other diseases ruled out, such as coronary heart disease with chest pain detected by coronary CTA examination, chronic renal insufficiency, and coronary heart disease. The study protocol was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Hainan Medical University, and all participants in the study signed informed consent. This study conformed to the principles outlined in the World Medical Association Declaration of Helsinki.

2.2. Methodology for clinical diagnosis

All subjects underwent two-dimensional echocardiography to measure left atrial diameter (LAD), left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic volume (LVEDV), left ventricle end-systolic volume (LVESV), and left ventricular ejection fraction (LVEF) determined according to the biplane Simpson method recommended by the American Society of Cardiac guidelines.

2.3. Genotyping

Venous blood (10 ml) was collected from all participants, standing 30 minutes and centrifuged at 3000 r/min for 20 minutes at 4 °C, and the supernatant plasma fraction was isolated divided into 2 samples and stored in a −80 °C refrigerator for testing. The genomic DNA was extracted from the leukocyte layer using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and stored at −80 °C. According to the gene sequence in the National Center for Biotechnology Information database, primer blast tool was used to design primers to amplify DNA segments including rs9722 locus. The primer sequence was: 5′-TCA GCT CCT ACT AGG CTG CAA-3′ (Forward); 5′-GAA AGC AGC CAA ACC TTT CCT-3′ (Reverse). The length of primers was 82 bp. The PCR reaction mixture contained 100 ng of genomic DNA, 2.5 μl of 10× buffer, 1.5 μl of Mg^{2+} (25 mM), 0.5 μl of dNTP (10 mM), 0.25 μl of Taq (5 U/ul), 1 μl of Forward and Reverse primers, and sterile water at a final volume of 25 μl. The PCR procedure was: pre-denaturation at 95 °C for 5 minutes, denaturation at 94 °C for 45 seconds; annealing at 56 °C for 45 seconds; extension at 72 °C for 30 seconds, and extension at 72 °C for 7 minutes after 30 cycles. The PCR products were sequenced by Sanger's method and compared with the sequence in the gene single nucleotide database (https://www.ncbi.nlm.nih.gov/snp/) to determine the genotype.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

One copy of plasma samples was taken out from a −80 °C refrigerator. Total RNA was extracted from plasma using the RNeasy mini kit (Qiagen, Valencia, CA), and cDNA was synthesized using the RevertAid First-Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. The primer sequences for hsa-miR-340-3p were: 5′-GCG GTT ATA AAG CAA TGA GA-3′ (Forward); 5′-GTG CGT GTC GTG GAG TCG-3′ (Reverse). The primer sequences for β-actin were: 5′-TGG CAC CAC ACC TTC TAC AAT-3′ (Forward); 5′-AGA GGC GTA CAG GGA TAG AGC A-3′ (Reverse). RT-PCR was conducted using a standard SYBR Green RT-PCR kit (Takara, Dalian, China) according to the manufacturer's instructions, the reaction was carried out in an ABI 7500 FAST Real-Time PCR System (Applied Biosystems), and the procedure was: denaturation at 94 °C for 5 minutes, followed by 30 cycles (94 °C, 30 seconds; 50 °C, 30 seconds; 72 °C, 30 seconds), and annealing at 72 °C for 10 minutes. The level of hsa-miR-340-3p in plasma relative to β-actin was analyzed by the $2^{-\Delta\Delta Ct}$ method. Each sample was measured three times.

2.5. Detection of plasma S100B level by enzyme-linked immunosorbent assay (ELISA)

The other copy of plasma samples was removed from −80 °C, melted on ice, and then the plasma S100B level was detected by a S100B detection kit (BioVendor L.M., A.S., Brno, Czech Republic). Plasma S100B protein levels were calculated using standard curve method. The detection range was 50 to 4000 pg/ml, with a sensitivity of 1 pg/ml, the variation between groups was <10%, and the intra-group variability was <10%. All operations are carried out in strict accordance with the supplier's instructions.

2.6. Statistical analyses

All categorical variables were expressed as a percentage [n(%)], and statistical analysis was performed using the χ^2 test. The Hardy-Weinberg equilibrium of *S100B* rs9722 locus was performed using the χ^2 test. Continuous variables were expressed as (mean ± SD), and statistical analyses were performed using t test. The association between SNP and the risk for CHF (ischemic cardiomyopathy (ICM) and dilated cardiomyopathy (DCM)) was determined based on the distribution of allele frequencies and

Table 1

General characteristics of patients with CHF and healthy controls.

	ICM (n = 124)	DCM (n = 91)	Control (n = 215)
Age (yr, mean ± SD)	63.47 ± 9.53	59.47 ± 7.99	61.08 ± 9.79
Gender[n (%)]			
Male	71 (57.26%)	52 (57.14%)	127 (59.07%)
Female	53 (42.74%)	39 (42.86%)	88 (40.93%)
BMI (kg/m^2, mean ± SD)	23.70 ± 2.95	23.40 ± 2.75	23.75 ± 3.08
SBP (mmHg, mean ± SD)	124.00 ± 5.93	126.12 ± 24.93	125.30 ± 11.83
DBP (mmHg, mean ± SD)	77.04 ± 11.21	75.87 ± 9.97	76.03 ± 11.20
HR (bpm, mean ± SD)	74.31 ± 10.28	75.75 ± 7.55	74.69 ± 13.33
LAD (mm, mean ± SD)	47.85 ± 9.74*	44.81 ± 10.58*	37.08 ± 7.75
LVEDD (mm, mean ± SD)	58.39 ± 9.81*	63.15 ± 15.08*	46.83 ± 9.73
LVESD (mm, mean ± SD)	51.71 ± 7.29*	49.21 ± 11.68*	29.26 ± 7.5
LVEDV (ml, mean ± SD)	210.61 ± 22.67*	218.77 ± 38.85*	111.84 ± 18.09
LVESV (ml, mean ± SD)	131.44 ± 22.67*	129.25 ± 13.55*	39.51 ± 14.01
LVEF (%, mean ± SD)	35.49 ± 7.54*	37.55 ± 5.41*	66.62 ± 14.87

BMI = body mass index, DBP = diastolic blood pressure, DCM = dilated cardiomyopathy, HR = heart rate, ICM = ischemic cardiomyopathy, LAD = left atrial diameter, LVEDD = left ventricular end-diastolic dimension, LVEDV = left ventricular end-diastolic volume, LVEF = left ventricular ejection fraction, LVESD = left ventricular end-systolic dimension, LVESV = left ventricular end-systolic volume, SBP = systolic blood pressure.

* $P < .05$ compared to the control group.

genetic models (additive, dominant, and recessive models), and age, gender, and BMI were adjusted using odds ratio (OR) and 95% confidence interval (CI) in unconditional logistic regression analysis. Association of hsa-miR-340-3p with S100B in plasma was analyzed using Pearson correlation. Statistical analyses were conducted using SPSS 22.0 (IBM, Chicago, IL), all tests were two-tailed, and $P < .05$ was considered statistically significant.

3. Results

3.1. General characteristics of study participants

Among the 215 patients with CHF, 124 had ICM and 91 had DCM. The general characteristics of patients with ICM, patients with DCM, and control groups are shown in Table 1. There was no significant difference in age, gender, BMI, systolic blood pressure, and diastolic blood pressure among the three groups ($P > .05$). While the LAD, LVEDD, LVESD, LVEDV, and LVESV were significantly higher, the LVEF was significantly lower in the patients with ICM and DCM than in the control group ($P < .05$).

3.2. Correlation between S100B rs9722 locus SNP and the risk for CHF

In the present study, the genotype distribution of the *S100B* rs9722 locus in all the subjects was determined using the Hardy-Weinberg equilibrium ($P = .18$; Table 2). Compared with the genotype frequency of CC, the genotype frequencies of CT and TT in patients with CHF were significantly higher than those of the control group ($P < .001$). We found that under additive, dominant, and recessive models, the risk of CHF increased by 1.56 times (95% CI: 1.15–2.10), 4.62 times (95% CI: 2.92–7.30), and 8.06 times (95% CI: 2.37–27.34), respectively. The risk of CHF was 4.24 times higher in subjects carrying the T allele than in those with the C allele (95% CI: 2.84–6.33, $P < .001$).

3.3. Stratified analysis for the association of S100B rs9722 SNP with CHF risk

We further analyzed the association between *S100B* rs9722 SNP and CHF risk at different age, gender, and BMI levels, and found that factors of age, gender, and BMI did not affect the association of s9722 SNP with CHF risk (Table 3). Meanwhile, we analyzed the association of *S100B* rs9722 locus SNP with ICM and DCM risk at different age, gender, and BMI levels, and found that age, gender, and BMI also did not influence the association of *S100B* rs9722 locus SNP with ICM and DCM risk (Table 4, Table 5).

3.4. Plasma S100B level

ELISA was performed to detect plasma S100B levels in all subjects. We found that the plasma S100B level was significantly

Table 2

Genotype and allele frequency of *S100B* rs9722 locus in CHF and control groups.

rs9722	CHF (n = 215)	Control (n = 215)	HWE *p*	Adjusted OR (95%CI)	*P*
CC	117 (54.42%)	182 (84.65%)	0.18	1.00 (reference)	
CT	76 (35.35%)	30 (13.95%)		3.94 (2.43–6.38)	**<.001**
TT	22 (10.23%)	3 (1.40%)		11.41 (3.34–38.97)	**<.001**
Additive model				1.56 (1.15–2.10)	**.004**
Dominant model				4.62 (2.92–7.30)	**<.001**
Recessive model				8.06 (2.37–27.34)	**<.001**
C	310 (72.09%)	394 (91.63%)		1.00 (reference)	
T	120 (27.91%)	36 (8.37%)		4.24 (2.84–6.33)	**<.001**

CHF = chronic heart failure, CI = confidence interval, HWE = Hardy-Weinberg equilibrium, OR = odds ratio.

Table 3

Stratified analysis for correlation between *S100B* rs9722 SNP and CHF risk.

	CHF (n = 215)	Control (n = 215)	Adjusted OR (95%CI)	*P*
Age (yr)				
≥60				
CC	47 (51.65%)	63 (86.30%)	1.00 (reference)	
CT/TT	44 (48.35%)	10 (13.70%)	5.90 (2.69–12.91)	<.001
<60				
CC	70 (56.45%)	119 (83.80%)	1.00 (reference)	
CT/TT	54 (43.55%)	23 (16.20%)	3.99 (2.26–7.06)	<.001
Gender				
Male				
CC	67 (54.47%)	106 (83.46%)	1.00 (reference)	
CT/TT	56 (45.53%)	21 (16.54%)	4.12 (2.35–7.59)	<.001
Female				
CC	50 (54.35%)	76 (86.36%)	1.00 (reference)	
CT/TT	42 (45.65%)	12 (13.64%)	5.32 (2.55–11.09)	<.001
BMI (kg/m^2)				
≥24				
CC	74 (56.49%)	109 (80.15%)	1.00 (reference)	
CT/TT	57 (43.51%)	27 (19.85%)	3.11 (1.80–5.36)	<.001
<24				
CC	43 (51.19%)	73 (92.41%)	1.00 (reference)	
CT/TT	41 (48.81%)	6 (7.59%)	11.60 (4.55–29.58)	<.001

BMI = body mass index, CHF = chronic heart failure, CI = confidence interval, OR = odds ratio.

Table 5

Stratified analysis for correlation between *S100B* rs9722 SNP and DCM risk.

	DCM (n = 91)	Control (n = 215)	Adjusted OR (95% CI)	*P*
Age (yr)				
≥60				
CC	13 (50.00%)	63 (86.30%)	1.00 (reference)	
CT/TT	13 (50.00%)	10 (13.70%)	6.30 (2.28–17.43)	<.001
<60				
CC	36 (55.38%)	119 (83.80%)	1.00 (reference)	
CT/TT	29 (44.62%)	23 (16.20%)	4.17 (2.15–8.08)	<.001
Gender				
Male				
CC	26 (50.00%)	106 (83.46%)	1.00 (reference)	
CT/TT	26 (50.00%)	21 (16.54%)	5.05 (2.46–10.34)	<.001
Female				
CC	23 (58.97%)	76 (86.36%)	1.00 (reference)	
CT/TT	16 (41.03%)	12 (13.64%)	4.41 (1.82–10.64)	.001
BMI (kg/m^2)				
≥24				
CC	32 (59.26%)	109 (80.15%)	1.00 (reference)	
CT/TT	22 (40.74%)	27 (19.85%)	2.78 (1.40–5.52)	.003
<24				
CC	17 (45.95%)	73 (92.41%)	1.00 (reference)	
CT/TT	20 (54.05%)	6 (7.59%)	14.31 (4.99–41.07)	<.001

BMI = body mass index, CI = confidence interval, DCM = dilated cardiomyopathy, OR = odds ratio.

higher in patients with ICM and DCM than in the control patients ($P < .001$); however, the S100B level was not significantly different between patients with ICM and DCM ($P > .05$; Fig. 1).

3.5. Association of S100B rs9722 SNP with plasma S100B levels

We further analyzed the association of plasma S100B levels in ICM, DCM, and control subjects with *S100B* rs9722 locus SNP and found that in ICM, DCM, and control subjects, the plasma S100B level was the lowest in subjects with the CC genotype, followed by the CT genotype, and the TT genotype subjects had the highest S100B level ($P < .05$; Fig. 2).

Table 4

Stratified analysis for correlation between *S100B* rs9722 SNP and ICM.

	ICM (n = 124)	Control (n = 215)	Adjusted OR (95% CI)	*P*
Age (yr)				
≥60				
CC	34 (52.31%)	63 (86.30%)	1.00 (reference)	
CT/TT	31 (47.69%)	10 (13.70%)	5.74 (2.52–13.12)	<.001
<60				
CC	34 (57.63%)	119 (83.80%)	1.00 (reference)	
CT/TT	25 (42.37%)	23 (16.20%)	3.80 (1.92–7.53)	<.001
Gender				
Male				
CC	41 (57.75%)	106 (83.46%)	1.00 (reference)	
CT/TT	30 (42.25%)	21 (16.54%)	3.69 (1.90–7.18)	<.001
Female				
CC	27 (50.94%)	76 (86.36%)	1.00 (reference)	
CT/TT	26 (49.06%)	12 (13.64%)	6.10 (2.71–13.75)	<.001
BMI (kg/m^2)				
≥24				
CC	42 (54.55%)	109 (80.15%)	1.00 (reference)	
CT/TT	35 (45.45%)	27 (19.85%)	3.36 (1.82–6.23)	<.001
<24				
CC	26 (55.32%)	73 (92.41%)	1.00 (reference)	
CT/TT	21 (44.68%)	6 (7.59%)	9.83 (3.58–27.03)	<.001

BMI = body mass index, CI = confidence interval, ICM = ischemic cardiomyopathy, OR = odds ratio.

3.6. Plasma hsa-miR-340-3p level

Furthermore, we analyzed the plasma level of hsa-miR-340-3p by RT-PCR. We found that the plasma hsa-miR-340-3p levels in patients with ICM and patients with DCM were significantly higher than those in the control group ($P < .001$). In addition, there was no significant difference in plasma hsa-miR-340-3p levels between patients with ICM and DCM ($P > .05$; Fig. 3).

3.7. Association of S100B rs9722 SNP with plasma hsa-miR-340-3p levels

The association of plasma hsa-miR-340-3p levels in ICM, DCM, and control subjects with *S100B* rs9722 locus SNP was also analyzed. We revealed that among ICM, DMC, and control groups, the plasma hsa-miR-340-3p levels were the lowest in subjects with the CC genotype, followed by the CT genotype, and the TT genotype had the highest hsa-miR-340-3p level ($P < .05$; Fig. 4).

3.8. Association of plasma hsa-miR-340-3p levels and S100B levels

We found that the plasma hsa-miR-340-3p levels were positively correlated with S100B levels in control subjects and patients with ICM and DCM (r = 0.61, 0.70, 0.81; respectively; Fig. 5A–C).

4. Discussion

With the improvements in living standards and alterations in diet structure and lifestyle, the incidence of cardiovascular diseases

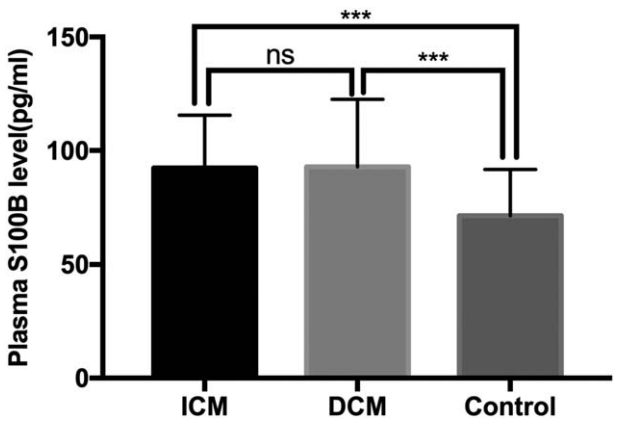

Figure 1. Comparison of plasma S100B levels in ICM, DCM, and control groups. ns = no significant; $^{***}P < .0001$.

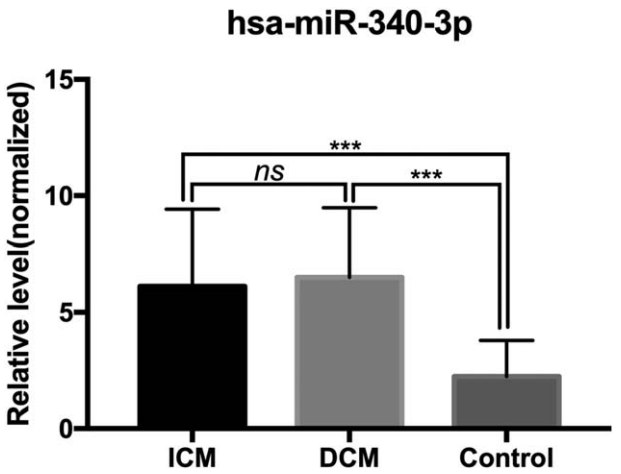

Figure 3. Comparison of plasma hsa-miR-340-3p levels in ICM, DCM, and control groups. ns = no significant; $^{***}P < .0001$.

has gradually increased in recent years, and the mortality rate has also increased significantly. S100B belongs to the S100 protein superfamily and has been shown to be a biomarker for central nervous system dysfunctions including cerebrovascular accidents, craniocerebral injury, mental disorders, and neonatal hypoxic encephalopathy.[15,16] Previously, a study revealed that rs9722 is a functional SNP in the 3′-UTR of *S100B*, which is closely related to the onset age of Parkinson disease.[12]

In the current study, we found that the *S100B* rs9722 locus SNP was associated with CHF risk in a Chinese Han population, and subjects carrying the T allele were 4.24 times more likely to have CHF than C allele carriers were. Further studies revealed that age, gender, and BMI factors did not affect the association of *S100B* rs9722 locus SNP with CHF risk, indicating that *S100B* rs9722 locus SNP was correlated with CHF risk regardless of different age, gender and BMI levels.

Moreover, we separated the patients with CHF into ICM and DCM groups to analyze the effect of age, gender, and BMI levels on the association of *S100B* rs9722 locus SNP with ICM and DCM risks. We found that age, gender, and BMI factors did not affect the association of *S100B* rs9722 locus SNP with ICM and DCM risks, indicating that *S100B* rs9722 locus SNP was correlated with ICM and DCM risks regardless of different age, gender, and BMI levels. The results of this study suggest that the general clinical characteristics of the subjects have a significant impact on the correlation between the SNP of *S100B* rs9722 and the risk of ICM and DCM, thus indicating that the occurrence of ICM and DCM is determined by genetic and environmental factors.

To investigate the underlying mechanisms of these correlations, we analyzed the plasma levels of S100B in all subjects. We found that among ICM, DCM, and control subjects, S100B level in the CC genotype was the lowest, followed by CT, and the TT genotype had the highest S100B level ($P < .05$), indicating that the plasma S100B level was higher in subjects carrying the T allele. Previous studies revealed that in children with hand, foot, and mouth disease (HFMD), the serum S100B level was the highest in *S100B* rs9722 TT genotype carriers, and the S100B level was the lowest in CC genotype carriers.[17] In addition, it was found that in healthy individuals, the S100B levels were significantly increased in *S100B* rs9722 locus T allele carriers,[18] which was consistent with the findings of the current study. We consider that the rs9722 locus is located in the 3'UTR region of the *S100B* gene. As we known that this region is the microRNA-gene binding site. Thus, this SNP may be located at the microRNA-S100B gene targeted binding site. The effect of microRNA on the expression regulation of S100B gene is specifically manifested as the change of S100B protein level, but it needs to be further verified in an in vitro cell model.

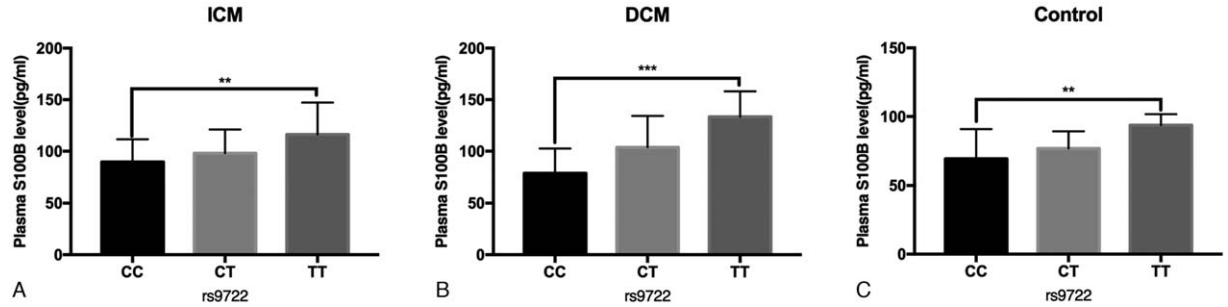

Figure 2. Plasma S100B levels in subjects with different genotypes of the *S100B* rs9722 locus. (A) Comparison of plasma S100B levels in different *S100B* rs9722 genotypes of patients with ICM. (B) Comparison of plasma S100B levels in different *S100B* rs9722 genotypes of patients with DCM. (C) Comparison of plasma S100B levels in different *S100B* rs9722 genotypes of healthy controls. $^{**}P < .001$; $^{***}P < .0001$.

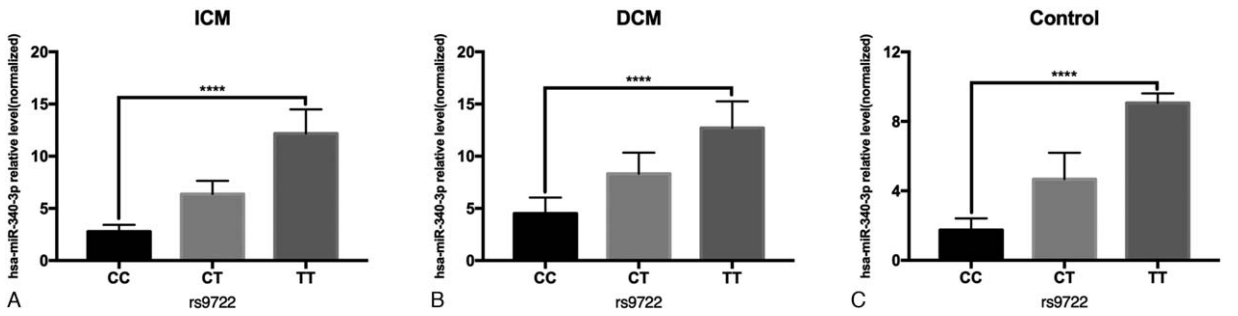

Figure 4. Plasma hsa-miR-340-3p levels in subjects with different genotypes at the *S100B* rs9722 locus. (A) Comparison of plasma hsa-miR-340-3p levels in different *S100B* rs9722 genotypes of patients with ICM. (B) Comparison of plasma hsa-miR-340-3p levels in different *S100B* rs9722 genotypes of patients with DCM. (C) Comparison of plasma hsa-miR-340-3p levels in different *S100B* rs9722 genotypes of controls. $^{****}P < .00001$.

Experimental evidence suggested that S100B is an intrinsic negative regulator of cardiac hypertrophy.[19] Usually, S100B is not expressed in normal myocardial tissue; however, it was found that the S100B expression is elevated in the heart and infarcted myocardium after myocardial infarction in humans. This was also found in the rat myocardium of a myocardial infarction model by ligation of coronary vessels.[20,21] In addition, previous studies found that the expression of S100B was significantly increased in depleted cardiomyocytes, it was negatively correlated with cardiac function, and may serve as a negative regulator in heart failure.[22,23]

To further investigate the reason for the higher level of S100B in *S100B* rs9722 locus T allele carriers, we screened out an miRNA which binds at the S100B rs9722 locus using the TargetScan tool (http://www.targetscan.org/mamm_31/), that is hsa-miR-340-3p. Hsa-mir-340-3p is encoded by the *MIR340* gene, which is located on the chromosome 5q35.3 (https://www.ncbi.nlm.nih.gov/gene/442908). We found that in the ICM, DCM, and control subjects, the plasma hs-miR-340-3p level was the lowest in CC genotype carriers, followed by the CT genotype, and TT genotype carriers had the highest hs-miR-340-3p level ($P < .05$), indicating that the level of hsa-miR-340-3p in the *S100B* rs9722 locus T allele carriers was higher. Further studies revealed that the level of hsa-miR-340-3p was positively correlated with the plasma level of S100B. Therefore, we speculate that hsa-miR-340-3p can up-regulate the expression of S100B, *S100B* rs9722 locus SNP affects the regulation of hsa-miR-340-3p on S100B expression, and T allele carriers have a higher S100B expression level. It is most likely that the S100B rs9722 locus T allele carriers have a higher binding efficiency of hsa-miR-340-3p and *S100B* gene, which promotes the expression of S100B protein; otherwise, the C allele carriers have a lower binding efficiency of hsa-miR-340-3p to the *S100B* gene, and the expression of the S100B protein is down-regulated.

However, there are few studies focused on the regulation of S100B expression by hsa-miR-340-3p, and there is no research evidence to support that hsa-miR-340-3p can regulate the expression of protein. Therefore, the major limitation of the current study is a lack of direct evidence to support the targeting of hsa-miR-340-3p to regulate the expression of S100B, in vitro models are needed to support this conclusion. Secondly, as an exploratory study, only the rs9722 locus was selected for analysis, and we cannot rule out the possibility that other functional SNP loci may also be associated with CHF risk. Thus, further studies are warranted. Additionally, further studies are needed to investigate the mechanism of S100B and its roles in heart failure. In addition, according to the allele frequency test results, the minimum sample size required for this study was 57 cases in the CHF group and 57 cases in the control group, and 215 cases and 215 cases were included in the CHF patients and control subjects in this study, which shows that the results of this study are objective.

In conclusion, *S100B* rs9722 locus SNP is associated with CHF risk in the Chinese Han population. *S100B* rs9722 locus T allele carriers have higher levels of S100B and hsa-miR-340-3p, and *S100B* rs9722 locus SNP may affect the regulation of S100B expression by hsa-miR-340-3p. In future work, we will study the correlation between the *S100B* rs9722 SNP and S100B protein expression and the mechanism of action of hsa-miR-340-3p in an in vitro cell model and verify it in a mouse model of chronic heart failure.

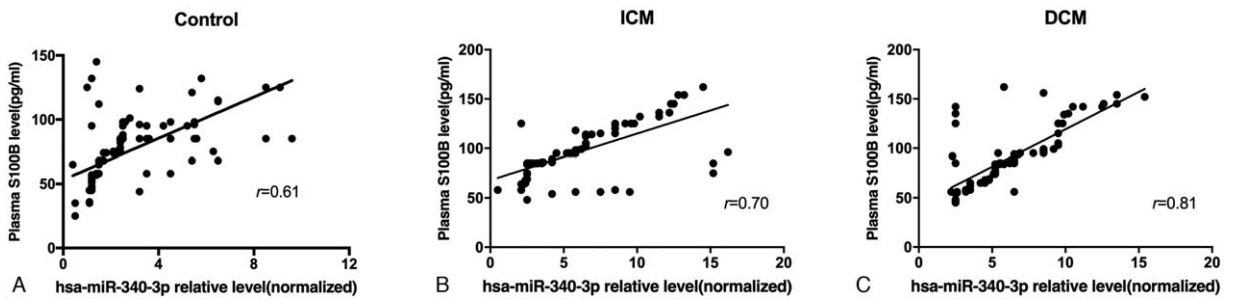

Figure 5. Correlation between plasma hsa-miR-340-3p levels and S100B levels. (A) The level of plasma hsa-miR-340-3p was correlated with the level of plasma S100B in control subjects. (B) The level of plasma hsa-miR-340-3p was correlated with the level of plasma S100B in patients with ICM. (C) The level of plasma hsa-miR-340-3p was correlated with the level of plasma S100B in patients with DCM.

Author contributions

YWC and XHC contributed equally to this work and shall share first authorship. YWC, XHC, and XXL conceived and designed the experiments. YWC, XHC, MZY, LC, and WWC performed the experiments. YWC and XHC analyzed the data. XXL wrote the paper. All authors read and approved the final manuscript.

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