

# The association of plasma connective tissue growth factor (CTGF) levels with hyperthyroidism heart disease: a cross-sectional observational study

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## Research article

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# Abstract

**Background:** Hyperthyroidism heart disease (HHD) is the most severe complication of overt hyperthyroidism and increases the mortality risk in affected patients. Early identification of patients at a higher risk of developing HHD could improve clinical outcomes through active surveillance and management. Connective tissue growth factor (CTGF), a secreted extracellular protein, plays a significant role in cardiac remodeling and dysfunction. We aimed to investigate the relationship between plasma CTGF level and the risk of HHD in this study.

**Methods:** A total of 142 overt hyperthyroidism patients without HHD and 99 patients with HHD were included. The plasma CTGF levels were measured via ELISA. Routine clinical medical data and echocardiography parameters were also recorded.

**Results:** The plasma CTGF level was significantly higher in patients with HHD than in those without HHD ( $P = 0.002$ ) and positively correlated with the levels of FT3, TRAb, TnI, LDH and the left atrium diameter, right atrium diameter, and right ventricular end-diastolic diameter (all  $P < 0.05$ ). Logistic regression analysis showed that quartile 3 and 4 of plasma CTGF levels were significantly associated with the increased risk of HHD (crude OR 2.529; 95% CI 1.188-5.387). However, after adjustment for the potentially confounding variables, only quartile 4 was significantly associated with the higher risk of HHD relative to quartile 1.

**Conclusions:** Hyperthyroidism patients with HHD display higher plasma CTGF levels. Furthermore, CTGF is an independent risk factor for HHD. Therefore, the plasma CTGF level may be a potential biomarker for the risk of HHD.

## Background

Overt hyperthyroidism, which is characterized by the excessive synthesis and secretion of thyroid hormones, is one of most common endocrine disease, affecting 0.2–1.3% of the people in iodine-sufficient places of the world[1, 2]. Excessive thyroid hormone secretion can markedly influence the cardiac function and induce structural changes, both directly and indirectly. Hyperthyroid heart disease (HHD) is the most severe complication that affects up to 20% of patients and is the leading cause of death in patients with overt hyperthyroidism[3–5]. Although studies have shown that effective treatment of hyperthyroidism can improve or reverse the cardiac dysfunction, structure alterations and arrhythmia in a majority of patients with HHD, persistent dilated cardiomyopathy was still existed in a significant proportion of patients[6]. Study showed that hyperthyroidism of some duration lead to physical cardiac hypertrophy through the activation of Akt in the early-stage disease that can revert after achieving euthyroid status. However, it converts to pathological later and even leads to heart failure in the late stage[7]. Ever if euthyroidism is achieved, the cardiac function or structure cannot normalize. Therefore, the early identification of patients at elevated risk of developing HHD could improve clinical outcomes

through enhanced surveillance and better management of hyperthyroidism. However, to our knowledge, there are no effective biomarkers to assess the risk of HHD in patients with overt hyperthyroidism.

Connective tissue growth factor (CTGF), a secreted extracellular protein, is a member of CNN family that has many kinds of biological effects, including the promotion of proliferation, angiogenesis, migration, extracellular matrix production etc, all of which are common features of cardiac remodeling. Studies have shown that various factors, such as transforming growth factor beta (TGF- $\beta$ )[8], angiotensin II (Ang II)[9], aldosterone[10], endothelin-1 (ET-1)[11], high glucose and free fatty acid[12], can modulate the expression of CTGF, but the relationship between the plasma CTGF levels and thyroid hormone is still unclear. Moreover, there is substantial evidence indicate that CTGF play an important role in the cardiac remodeling and function[13–16]. Vitro experiments have shown that CTGF can not only cause cardiomyocyte hypertrophy, but also promote cardiac fibroblasts proliferation and deposition of extracellular matrix (ECM)[9, 12, 17]. Interestingly, as in the pathogenesis of HHD, studies demonstrated that CTGF induced cardiomyocyte hypertrophy via Akt pathway but not MAPK pathway and didn't upregulate skeletal actin and brain natriuretic peptide (BNP) mRNA, which are biomarkers of pathological cardiac hypertrophy[17]. Additionally, in combination with Akt activation, CTGF overexpression initially leads to cardiac hypertrophy, which is followed by ventricular dilatation for a longer time in vivo[18]. Moreover, a recent study showed that high levels of CTGF can predict the risk of future myocardial infarction (MI) and cardiovascular death in patients with type 2 diabetes[19]. However, the circulating CTGF levels have not been investigated in hyperthyroidism patients with or without HHD .

Therefore, we aimed to examine the plasma levels of CTGF in hyperthyroidism patients with or without HHD, and explore the relationship between plasma CTGF levels and the risk of HHD in present study.

## Methods

### Research subjects

We enrolled 241 patients who were diagnosed with overt hyperthyroidism between May 2018 and May 2019 in the inpatient department of endocrinology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. As defined in the guidelines of the American Thyroid Association Guidelines, overt hyperthyroidism was defined as increased free thyroxine (FT4) and/or free triiodothyronine (FT3) level and a concomitantly suppressed thyroid-stimulating hormone (TSH) level[20]. HHD was diagnosed in patients with overt hyperthyroidism with at least one of the following cardiac abnormalities: arrhythmia (e.g., atrial fibrillation, paroxysmal supraventricular tachycardia, frequent ventricular premature beat, conduction block, etc.), heart failure, cardiac dilatation, angina pectoris or MI and valve prolapse with pathological murmur in the cardiac auscultation area[21, 22]. In addition, patients with cardiac disease secondary to other etiologies should be excluded.

### Exclusion criteria

We excluded subjects with conditions, including tumor, sepsis, severe injury, diabetes mellitus, hyperlipidaemia, atherosclerosis, chronic renal failure and fibrosis-associated disease, known to be associated with an increased circulating CTGF level. In addition, we excluded patients with a history of surgery within 6 months prior to admission.

### **Blood sampling and measurement of CTGF**

Venous blood samples were drawn into collection tubes containing EDTA in the morning of the second day following the admission and after at least 8 hours overnight fasting. The plasma was separated from blood cells within 2 hours of sample collection by centrifugation at 2500 g for 15 minutes at room temperature, and pipette out and frozen at -80°C until the assay with an ELISA kit.

### **Medical data collection of subjects**

Data on the demographic characteristics, physical examinations, routine biochemical marker, thyroid function tests, radioactive iodine uptake test and <sup>99m</sup>Tc sodium pertechnetate thyroid uptake test were recorded, including age, sex, weight, height, heart rate, blood pressure, duration of symptoms of hyperthyroidism, estimated thyroid mass and the levels of total bilirubin (TBIL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum albumin (ALB), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), uric acid (UA), creatinine (Cr), blood uric nitrogen (BUN), triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), fasting plasma glucose (FPG), serum electrolytes (i.e., sodium, potassium, calcium, phosphorus, magnesium), BNP, creatine kinase (CK), creatine kinase-MB (CK-MB), cardiac troponin I (TnI), lactate dehydrogenase (LDH), TSH, FT4, FT3, anti-thyroglobulin antibody (TGAb), anti-thyroid peroxidase antibody (TPOAb), thyrotropin receptor antibody (TRAb), and 2-hour and 24-hour radioactive iodine (<sup>131</sup>I) uptake (RAIU). The body mass index (BMI) was calculated as the weight (kg) divided by height (m) squared.

### **Transthoracic echocardiography**

A complete transthoracic echocardiography (TTE) was undertaken in all participants by using an ultrasonographic system with a 3~8 MHz transducer (GE Vivid 7; Vingmed; Philips EPIQ 7C and Philips IE33), in accordance with the recommendations of the American Society of Echocardiography[23]. All examinations were conducted and analyzed by an experienced operator who was unaware of the patients' clinical status. The following parameters were recorded on TTE: the ascending aorta diameter (AAOD), left ventricular end-diastolic diameter (LVEDD), interventricular septum thickness (IVST), right ventricular end-diastolic diameter (RVEDD), right left atrium diameter (LAD), atrium diameter (RAD), pulmonary artery diameter (PAD); left ventricular ejection fraction (LVEF). In addition, the peak velocities of the early (E-wave) (MVE) and late (A-wave) (MVA) phase of the mitral inflow pattern were measured from an apical four-chamber view. Moreover, we ascertained whether the patients had pulmonary hypertension (PH), left ventricular (LV) diastolic dysfunction and valvular regurgitation according to the recommendations[24-26]. The pulmonary arterial systolic pressure was estimated from the sum of the

tricuspid regurgitation maximal velocity and estimated right atrial pressure by using the simplified Bernoulli equation. PH was defined as a mean pulmonary artery that was greater than or equal to 25 mm Hg. LV diastolic dysfunction was defined by the mitral E/A ratio or septal basal regions e/a ratio less than 1. Color flow doppler was used for the detection of valvular regurgitation.

## Statistical Analysis

All statistical analyses were conducted with SPSS 22.0 (SPSS, Chicago, Illinois, USA). The Kolmogorov-Smirnov test was conducted to confirm whether the continuous variables conformed to normal distribution. The patients' data were expressed as mean  $\pm$  standard deviation (SD) for normally distributed data and median (interquartile range [IQR] 25th–75th percentile) for data with skewed distribution data. All categorical variables were presented as numbers (proportions). The intergroup differences were analyzed by the Student's t-test, the Mann-Whitney test, or the Chi-square test, as appropriate. Spearman's correlation analysis was undertaken to explore the associations between the CTGF level and thyroid-related and echocardiography parameters. A binary logistic regression analysis was conducted to identify odds ratios (OR) and 95% confidence intervals (CI) of HHD by quintiles of the plasma CTGF levels. Additionally, potential confounders were controlled. All tests were two-sided, and  $P$ -values  $< 0.05$  were considered statistically significant.

## Results

### Clinical and biochemical characteristics of the subjects

The characteristics of the hyperthyroidism patients with or without HHD are shown in Table 1. A total of 241 patients with hyperthyroidism (with HHD,  $n = 142$ ; without HHD,  $n = 99$ ) were included in the final analysis dataset in this study. Compared to those without HHD, the patients with HHD were older; had significantly higher levels of SBP, TBIL, GGT, BNP, TNI, LDH, FT4, TPOAb, and TRAb; and significantly lower levels of ALB, LDL-C, K, Ca, and P (all  $P$ -values  $< 0.05$ ). Besides these, the patients with HHD tended to have higher BMI ( $P = 0.08$ ), higher 2-hour RAIU ( $P = 0.067$ ) and lower levels of serum Cr ( $P = 0.057$ ) than did patient without HHD, but the differences were not statistically significant.

### Comparison of echocardiographic parameters between hyperthyroidism patients with or without HHD

The characteristics of echocardiographic parameters are shown in Table 2. AAOD, LAD, LVEDD, RAD, RVEDD, PAD, and MVA were significantly higher in patients with HHD than in those without HHD. Additionally, the incidences of PH, LV diastolic dysfunction, mitral regurgitation, tricuspid regurgitation and aortic regurgitation were higher in patients with HHD (all  $P$ -values  $< 0.05$ ). Nevertheless, there were no significant differences in IVST, LVEF and MVE between patients with and without HHD.

### Comparison of circulating plasma CTGF levels between patients with or without HHD

The plasma CTGF levels were significantly higher in patients with HHD [8.29 (6.91-9.30) ng/ml] than in those without (7.52 [6.00–8.87] ng/mL;  $P = 0.002$ ) (Figure.1).

## **Correlations of CTGF with thyroid related examinations and continuous parameters associated with cardiac**

Correlations between the plasma CTGF levels and thyroid-related and continuous parameters associated with cardiac function are shown in Table 3 and Table 4, respectively. The levels of FT3 and TRAb both positively correlated with the plasma CTGF levels, whereas the levels of FT4, TSH, TPOAb, TgAb, 2-hour RAIU, 24-hour RAIU, and thyroid mass did not significantly correlate with plasma CTGF levels. With regard to the biochemical cardiac parameters, the plasma CTGF levels were significantly positively correlated with LDH, TNI. For the echocardiography parameters, the plasma CTGF levels were positively correlated with LAD, RAD and RVEDD, but there were no correlations with LVEF or BNP. Moreover, the plasma CTGF levels were positively but not significantly correlated with MVA.

## **The associations between plasma CTGF levels and HHD in the logistic regression analysis**

The odds ratios (OR) and 95% confidence interval (CI) for the associations between plasma CTGF levels and HHD in the logistic regression analysis are shown in Table 5. In the unadjusted Model 1, compared with quartile 1 of CTGF levels, quartile 3 and 4 were significantly associated with presence of HHD, and the significance persisted after adjustment for age and sex. When the duration of symptoms of hyperthyroidism and BMI were further included in Model 3, the above mentioned trend persisted. However, after further adjustment for the related factors of HHD, including HR, SBP, DBP, FT4, TSH, TRAb, and TPOAb, we found that quartile 3 of CTGF levels was no longer associated with the presence of HHD, but the trend of quartile 4 remained unchanged.

## **Discussion**

The plasma CTGF levels were significantly higher in hyperthyroidism patients with HHD than those without HHD. Moreover, a higher CTGF level was an independent risk factor for HHD after adjustment for potential confounders, thereby indicating an association between the plasma CTGF levels and the risk of HHD.

CTGF is a secreted extracellular protein and has been implicated to play a role in the processes of inflammatory, response to injury and wound repair[27]. Increasingly, evidence has shown that CTGF is a pathogenic risk factor for cardiac diseases. Not only can CTGF induce cardiomyocytes hypertrophy, but also it can promote proliferation of cardiac fibroblasts in vitro[9, 12]. Studies have shown that the expression of CTGF was increased in cardiac tissue of patients with cardiomyopathy or heart failure[9, 16]. In addition, a study recently demonstrated that the plasma levels of CTGF were higher in patients with diabetes who were susceptible to MI and cardiovascular events[19]. All of the above mentioned aspects indicate that CTGF plays an important role in the pathogenesis of cardiac disease, Whereas, the relationship between the plasma CTGF levels and risk of HHD in human remains unknown.

Our current study demonstrated for the first time that the CTGF levels were higher in overt hyperthyroidism patients with HHD than those without HHD and that a higher CTGF level was an

independent risk factor for HHD. It is generally known that the thyroid hormones directly affect cardiac function and structure through a hormonal effect on the heart, by regulating the expression of various genes that are related to myocyte contraction or rhythm after binding to the thyroid hormone-response elements; the indirect effects are related to the altered LV loading conditions[28]. Besides the classical action, studies have demonstrated that thyroid hormones induced cardiac hypertrophy partially via activating Akt signaling pathway[29]. Furthermore, Hayata et al. showed that CTGF induced cardiac hypertrophy through Akt signaling [7, 17]. Additionally, studies have demonstrated that inhibition of CTGF can alleviate or reverse cardiac fibrosis, which was similar to the development of HHD[16, 30]. After achievement of a euthyroid state, the cardiac function or structure may revert to normal in patients with HHD. In the hyperthyroidism state, the reduction in systemic vascular resistance results in decreased renal perfusion pressure, with the resultant activation of the renin-angiotensin-aldosterone system (RAAS). Moreover, there is evidence that T3 can stimulate the hepatic synthesis of rennin substrate and increase the cardiac expression of rennin, both of which could potentially contribute to elevated levels of rennin and Ang II[4]. Studies have shown that various factors can increase the expression of CTGF, including Ang II, aldosterone. Besides, Studies have shown that they induced cardiac disease by activation of Akt signaling. Suggesting that CTGF may be one of the mediator of thyroid hormones on heart and may play an important role in cardiac dysfunction in patients with hyperthyroidism. However, the specific mechanism of the action of thyroid hormones on the CTGF expression and whether it occurs via or indirect pathways need be further investigated.

Despite a number of factors that have been implicated in the regulation of the CTGF expression in cardiac tissue, the association between the plasma CTGF levels and thyroid hormones or thyroid-related antibody have not been explored previously. In this study, we found that the plasma CTGF levels positively correlated with the levels of FT3 and TRAb. Similarly, Huang et al. reported that the levels of CTGF expression in orbital fibroblasts in Graves' ophthalmopathy showed a significant positive correlation with serum levels of TRAb; however, a correlation between the levels of CTGF expression and FT3 were not be found[31]. Although the above mentioned results do not absolutely match those in this study, the circulating levels of CTGF may be more representative of systemic conditions than that in orbital fibroblasts. TRAb is one type of thyroid autoantibodies, which contributes to the excessive increase of FT3 and FT4 that has been identified as the main cause of hyperthyroidism and is associated with disease remission. In addition, FT3 has genomic action and can activate or repress the expression of a variety of genes by binding to thyroid hormone response elements[28]; moreover, FT3 can activate the RASS, with the resultant elevated expression of CTGF. Therefore, TRAb may affect the levels of CTGF due to changes in the serum levels of thyroid hormone. Furthermore, TRAb can induce an autoimmune response, thereby potentially implicating an immune and mechanism that needs to be further investigated. Thus, we speculated that FT3 or TRAb may promote the expression of CTGF via direct or indirect ways mechanisms, and their action on CTGF expression should be further studied in vitro and in vivo.

Previous studies demonstrated that CTGF can contribute to cardiomyocytes hypertrophy and increased extracellular matrix, which initially induce cardiac hypertrophy and eventually heart failure[9, 18]. The

research conducted by Chen suggested that CTGF expression levels in the atrial tissue of patients with atrial fibrillation were significantly increased and positively correlated with LAD and duration of atrial fibrillation [32]. In this study, we found that the plasma CTGF levels were positively correlated with LAD, RAD and RVEDD, implicating CTGF in cardiac remodeling and as a potential mediator for the action of the thyroid hormone on cardiac tissues. Furthermore, we found that the plasma CTGF levels were positively correlated with TNI and LDH. Therefore, CTGF may be also a biomarker indicating myocardial impairment. However, the CTGF levels were not significantly correlated with BNP, which is in agreement with the results reported by Hayata et al[17]. The reason may be that CTGF doesn't upregulate the expression of BNP to some extent or the pathological disease condition of included participants was relatively mild.

Despite being the first study to explore the plasma CTGF levels in overt hyperthyroidism patients and to evaluate their association with cardiac dysfunction. The study has some limitations. The cross-sectional observational study design prevents the determination of the causality of CTGF in the development of HHD in patients with hyperthyroidism. Therefore, the causal effect of CTGF on HHD needs to be evaluated in a cohort study. Next, we only enrolled Chinese study participants, and a control group of normal participants was not established. Therefore, further research to examine the plasma CTGF levels in healthy individuals or in other ethnic populations is needed. Moreover, the sample size of this study was relatively small.

## Conclusions

In summary, the data from this study indicate that higher plasma CTGF levels are associated with the presence of HHD in hyperthyroidism patients. Furthermore elevated plasma CTGF levels were associated with increased LAD, RAD, and RVEDD in patients with overt hyperthyroidism. Therefore, the plasma CTGF levels may be a potential biomarker for assessing the risk of HHD and facilitate better monitoring and management of patients with hyperthyroidism who are at risk of HHD. There is a need for further research to elucidate the specific mechanisms of the action of the thyroid hormone on CTGF expression and the role of CTGF in the development of HHD.

## Declarations

### Ethics approval and consent to participate

Approval was obtained from the Tongji Medical College of Huazhong University of Science and Technology, and the procedures used in this study adhere to the tenets of the Declaration of Helsinki. All enrolled subjects signed the informed consent agreement.

### Consent for publication

Not applicable.

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## Competing interests

All authors declare that they have no conflicts of interest.

## Data availability statement

The data that support the findings of this study are available from the corresponding author on reasonable request.

## Authors' contributions

All authors critically reviewed and edited the manuscript and approved the final version for submission. LYM, WLF, LYF conceived the study. LYM, WLF, LH developed the study design and methods. LH, ZRL collected the data, LH conducted the assay, data analysis and wrote the manuscript. FMF, ZH researched the data.

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## Abbreviations

CTGF: connective tissue growth factor; HHD: hyperthyroid heart disease; BW: body weight; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; TBIL: total bilirubin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALB: albumin; ALP: alkaline phosphatase; GGT: gamma-glutamyl transpeptidase; Cr: creatinine; BUN: blood uric nitrogen; UA: uric acid; TC: total cholesterol; TG: triglyceride; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; FPG: fasting plasma glucose; Na: sodium; K: potassium; Ca: total serum calcium; P: phosphorus; Mg: magnesium; BNP: brain natriuretic peptide; CK: creatine kinase; CK-MB: creatine kinase-MB; Tnl: cardiac troponin I; LDH: lactate dehydrogenase; FT3: free triiodotronine; FT4: free thyroxine; TSH: thyroid stimulating hormone; TPOAb: anti-thyroid peroxidase antibody; TGAb: anti-thyroglobulin antibody; TRAb: thyrotropin receptor antibody; RAIU radioactive iodine (131I) uptake; AAO: ascending aorta diameter; PAD: [pulmonary artery](#) diameter; LAD: left atrium diameter; LVEDD: left ventricular end-diastolic diameter; IVST: interventricular septum thickness; RAD: right atrium diameter; RVEDD: right ventricular end-diastolic diameter; LVEF: left ventricular ejection fraction; MVE: peak velocities of the early (E-wave) phase of the mitral inflow pattern; MVA: peak velocities of the late (A-

wave) phase of the mitral inflow pattern; PH: pulmonary hypertension; OR: odds ratios; CI: confidence interval; Ref: reference; SD: standard deviation.

## References

1. Taylor PN, Albrecht D, Scholz A, Gutierrez-Buey G, Lazarus JH, Dayan CM, et al. Global epidemiology of hyperthyroidism and hypothyroidism. *Nat Rev Endocrinol*. 2018;14(5):301–16.
2. Brandt F, Thvilum M, Almind D, Christensen K, Green A, Hegedus L, et al. Morbidity before and after the diagnosis of hyperthyroidism: a nationwide register-based study. *PLoS One*. 2013;8(6):e66711.
3. Brandt F, Green A, Hegedus L, Brix TH. A critical review and meta-analysis of the association between overt hyperthyroidism and mortality. *European journal of endocrinology*. 2011;165(4):491–7.
4. Osuna PM, Udovcic M, Sharma MD. Hyperthyroidism and the Heart. *Methodist Debaquey Cardiovasc J*. 2017;13(2):60–3.
5. Jabbar A, Pingitore A, Pearce SH, Zaman A, Iervasi G, Razvi S. Thyroid hormones and cardiovascular disease. *Nat Rev Cardiol*. 2017;14(1):39–55.
6. Siu CW, Yeung CY, Lau CP, Kung AW, Tse HF. Incidence, clinical characteristics and outcome of congestive heart failure as the initial presentation in patients with primary hyperthyroidism. *Heart*. 2007;93(4):483–7.
7. Dillmann W. Cardiac hypertrophy and thyroid hormone signaling. *Heart Fail Rev*. 2010;15(2):125–32.
8. Chen MM, Lam A, Abraham JA, Schreiner GF, Joly AH. CTGF expression is induced by TGF- $\beta$  in cardiac fibroblasts and cardiac myocytes: a potential role in heart fibrosis. *J Mol Cell Cardiol*. 2000;32(10):1805–19.
9. Ahmed MS, Oie E, Vinge LE, Yndestad A, Oystein Andersen G, Andersson Y, et al. Connective tissue growth factor—a novel mediator of angiotensin II-stimulated cardiac fibroblast activation in heart failure in rats. *J Mol Cell Cardiol*. 2004;36(3):393–404.
10. Messaoudi S, Gravez B, Tarjus A, Pelloux V, Ouvrard-Pascaud A, Delcayre C, et al. Aldosterone-specific activation of cardiomyocyte mineralocorticoid receptor in vivo. *Hypertension*. (Dallas, Tex: 1979). 2013;61(2):361-7.
11. Recchia AG, Filice E, Pellegrino D, Dobrina A, Cerra MC, Maggiolini M. Endothelin-1 induces connective tissue growth factor expression in cardiomyocytes. *J Mol Cell Cardiol*. 2009;46(3):352–9.
12. Wang X, McLennan SV, Allen TJ, Tsoutsman T, Semsarian C, Twigg SM. Adverse effects of high glucose and free fatty acid on cardiomyocytes are mediated by connective tissue growth factor. *Am J Physiol Cell Physiol*. 2009;297(6):C1490-500.
13. Chi H, Feng H, Shang X, Jiao J, Sun L, Jiang W, et al. Circulating Connective Tissue Growth Factor Is Associated with Diastolic Dysfunction in Patients with Diastolic Heart Failure. *Cardiology*. 2019;143(3–4):77–84.
14. Koitabashi N, Arai M, Niwano K, Watanabe A, Endoh M, Suguta M, et al. Plasma connective tissue growth factor is a novel potential biomarker of cardiac dysfunction in patients with chronic heart

- failure. *Eur J Heart Fail.* 2008;10(4):373–9.
15. Tan CY, Wong JX, Chan PS, Tan H, Liao D, Chen W, et al. Yin Yang 1 Suppresses Dilated Cardiomyopathy and Cardiac Fibrosis Through Regulation of Bmp7 and Ctgf. *Circ Res.* 2019;125(9):834–46.
  16. Koshman YE, Sternlicht MD, Kim T, O'Hara CP, Koczor CA, Lewis W, et al. Connective tissue growth factor regulates cardiac function and tissue remodeling in a mouse model of dilated cardiomyopathy. *J Mol Cell Cardiol.* 2015;89(Pt B):214–22.
  17. Hayata N, Fujio Y, Yamamoto Y, Iwakura T, Obana M, Takai M, et al. Connective tissue growth factor induces cardiac hypertrophy through Akt signaling. *Biochem Biophys Res Commun.* 2008;370(2):274–8.
  18. Panek AN, Posch MG, Alenina N, Ghadge SK, Erdmann B, Popova E, et al. Connective tissue growth factor overexpression in cardiomyocytes promotes cardiac hypertrophy and protection against pressure overload. *PLoS One.* 2009;4(8):e6743.
  19. Hunt KJ, Jaffa MA, Garrett SM, Luttrell DK, Lipson KE, Lopes-Virella MF, et al. Plasma Connective Tissue Growth Factor (CTGF/CCN2) Levels Predict Myocardial Infarction in the Veterans Affairs Diabetes Trial (VADT) Cohort. *Diabetes Care.* 2018;41(4):840–6.
  20. Ross DS, Burch HB, Cooper DS, Greenlee MC, Laurberg P, Maia AL, et al. 2016 American Thyroid Association Guidelines for Diagnosis and Management of Hyperthyroidism and Other Causes of Thyrotoxicosis. *Thyroid.* 2016;26(10):1343–421.
  21. Zhang X, Chen L, Sheng J, Li C, He Y, Han W. The Association of Autoantibodies in Hyperthyroid Heart Disease Combined with Pulmonary Hypertension. *Int J Endocrinol.* 2019;2019:9325289.
  22. Wang Q, Li C, Di S, Han L, Zhao L, Tong X. Clinical efficacy and safety of traditional Chinese patent medicine for hyperthyroid heart disease: study protocol for a systematic review and meta-analysis. *Medicine.* 2018;97(45):e13076.
  23. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr.* 2015;28(1):1–39 e14.
  24. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF 3rd, Dokainish H, Edvardsen T, et al. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr.* 2016;29(4):277–314.
  25. Zoghbi WA, Enriquez-Sarano M, Foster E, Grayburn PA, Kraft CD, Levine RA, et al. Recommendations for evaluation of the severity of native valvular regurgitation with two-dimensional and Doppler echocardiography. *J Am Soc Echocardiogr.* 2003;16(7):777–802.
  26. Galie N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the

- European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Respir J*. 2015;46(4):903–75.
27. Jun JI, Lau LF. Taking aim at the extracellular matrix: CCN proteins as emerging therapeutic targets. *Nat Rev Drug Discov*. 2011;10(12):945–63.
28. Vargas-Uricoechea H, Bonelo-Perdomo A, Sierra-Torres CH. Effects of thyroid hormones on the heart. *Clin Investig Arterioscler*. 2014;26(6):296–309.
29. Carneiro-Ramos MS, Diniz GP, Nadu AP, Almeida J, Vieira RL, Santos RA, et al. Blockage of angiotensin II type 2 receptor prevents thyroxine-mediated cardiac hypertrophy by blocking Akt activation. *Basic Res Cardiol*. 2010;105(3):325–35.
30. Lipson KE, Wong C, Teng Y, Spong S. CTGF is a central mediator of tissue remodeling and fibrosis and its inhibition can reverse the process of fibrosis. *Fibrogenesis Tissue Repair*. 2012;5(Suppl 1):24.
31. Huang YM, Chang PC, Wu SB, Kau HC, Tsai CC, Liu CJ, et al. Expression and clinical significance of connective tissue growth factor (CTGF) in Graves' ophthalmopathy. *Br J Ophthalmol*. 2017;101(5):676–80.
32. Chen JQ, Guo YS, Chen Q, Cheng XL, Xiang GJ, Chen MY, et al. TGFbeta1 and HGF regulate CTGF expression in human atrial fibroblasts and are involved in atrial remodelling in patients with rheumatic heart disease. *J Cell Mol Med*. 2019;23(4):3032–9.

## Tables

**Table 1** Characteristics of patients with and without HHD

	All (N = 241)	Without HHD (N = 142)	With HHD (N = 99)	P-value
Age (years)***	36.5 (27.9-48.4)	31.8 (26.8-45.6)	43.3 (32.1-50.3)	<0.001
Duration (years)	2.00 (0.17-6.00)	2.00 (0.17-5.00)	2.00 (0.17-7.00)	0.777
Male, n (%)	80 (33.2)	53 (37.3)	27 (27.3)	0.103
BW (kg)	55 (50-61)	55 (50-60)	55 (49-62)	0.875
BMI (kg/m <sup>2</sup> )	20.8 (19.1-22.7)	20.4 (19.0-21.9)	21.1 (19.1-23.4)	0.080
SBP (mmHg) *	127 ± 15	125 ± 15	129 ± 15	0.030
DBP (mmHg)	76 (70-84)	76 (70-84)	78 (70-85)	0.504
HR (beats/min)	95 (81-105)	95 (81-107)	93 (80-104)	0.485
TBIL (umol/L) ***	12.5 (10.0-16.5)	12.1 (9.6-14.6)	15.3 (11.0-19.2)	<0.001
ALT (U/L)	31 (20-44)	33 (21-46)	28 (18-40)	0.115
AST (U/L)	25 (19-32)	24 (18-33)	25 (21-32)	0.125
ALB (g/L) ***	39.5 ± 3.5	40.3 ± 3.1	38.4 ± 3.6	<0.001
ALP (U/L)	106 (70-122)	106 (73-110)	96 (68-147)	0.581
GGT (U/L) *	27 (16-32)	31 (20-32)	22 (15-31)	0.021
Cr (umol/L)	42.1 (35.8-52.0)	43.0 (37.7-52.3)	39.4 (33.3-50.0)	0.057
BUN (mmol/L)	4.58 (3.59-5.56)	4.50 (3.57-5.51)	4.60 (3.61-5.80)	0.637
UA (umol/L)	316.9 ± 84.9	314.2 ± 81.4	320.8 ± 90.0	0.555
TC (mmol/L)	2.99 (2.63-3.25)	2.99 (2.80-3.22)	2.98 (2.51-3.34)	0.098
TG (mmol/L)	0.92 (0.71-1.06)	0.92 (0.70-1.00)	0.90 (0.71-1.08)	0.664
HDL (mmol/L)	1.16 (0.95-1.31)	1.16 (0.99-1.28)	1.13 (0.91-1.37)	0.653
LDL (mmol/L) *	1.53 (1.27-1.69)	1.53 (1.39-1.72)	1.44 (1.20-1.67)	0.022
FPG (mmol/L)	4.85 (4.65-5.07)	4.85 (4.67-5.00)	4.85 (4.63-5.13)	0.295
Na (mmol/L)	140.9 ± 1.8	140.7 ± 1.8	141.1 ± 1.9	0.108
K (mmol/L) ***	3.95 ± 0.35	4.02 ± 0.34	3.85 ± 0.33	<0.001
Ca (mmol/L) **	2.29 ± 0.13	2.32 ± 0.12	2.26 ± 0.13	0.001
P (mmol/L) **	1.20 ± 0.24	1.24 ± 0.22	1.15 ± 0.25	0.002
Mg (mmol/L)	0.81 (0.75-0.86)	0.82 (0.76-0.86)	0.80 (0.74-0.85)	0.187
BNP (pg/ml) ***	74.4 (26.3-175.3)	27.9 (14.0-75.3)	129.7 (63.9-268.8)	<0.001
CK (U/L)	51.0 (36.0-60.2)	53.5 (37.0-60.2)	47.0 (33.0-60.2)	0.200
CK-MB (U/L)	20 (17-24)	20 (16-23)	21 (17-26)	0.158
LDH (U/L) *	169 (151-185)	166 (150-180)	171 (153-195)	0.011
TnI (ng/L) **	2.6 (1.3-5.5)	1.4 (0.7-4.1)	2.9 (2.3-5.9)	0.007
FT3 (pmol/L)	27.56 (16.83-36.52)	26.52 (16.61-35.25)	29.03 (17.58-39.60)	0.205
FT4 (pmol/L) **	65.59 (45.38-99.95)	60.54 (42.76-91.64)	73.11 (40.30-100.00)	0.008
TSH (mIU/L)				0.786
<0.005, n (%)	173 (71.8)	101 (71.1)	72 (72.7)	
≥0.005, n (%)	68 (28.2)	41 (28.9)	27 (27.3)	
TPOAb (IU/ml) **	270.00 (88.55-451.25)	225.00 (46.31-375.98)	291.50 (154.00-564.30)	0.003
TgAb (IU/ml)	189.10 (19.89-549.23)	149.55 (27.13-549.23)	201.60 (16.48-549.23)	0.680
TRAb (IU/L) ***	13.43 (5.70-24.80)	10.42 (4.96-17.83)	17.10 (9.24-34.30)	<0.001
2-h RAIU	51.6 ± 19.0	49.0 ± 19.8	55.3 ± 17.3	0.067

24-h RAIU	70.4 (63.2-76.5)	69.9 (61.7-76.3)	70.6 (64.7-77.4)	0.526
Thyroid mass (g)	63.8 (50.4-84.5)	63.3 (50.2-81.8)	63.8 (50.3-93.0)	0.546

Normal distributed continuous variables were presented as mean  $\pm$  SD. Skewed distributed continuous variables were presented as median (interquartiles). Categorical variables were presented as number (proportions). Differences between groups were analyzed by using Student's t-test, Mann-Whitney test, or Chi-square test, as appropriate

\*  $P$  value  $< 0.05$ , \*\*  $P$  value  $< 0.01$ , \*\*\*  $P$  value  $< 0.001$

**Table 2** Comparison of echocardiographic parameters between hyperthyroidism patients with or without HHD

	Without HHD (N = 142)	With HHD (N = 99)	$P$ -value
	(N=142)		
AAOD (cm)***	2.8 (2.7-3.0)	3.0 (2.8-3.2)	$<0.001$
PAD (cm)***	2.3 (2.1-2.5)	2.4 (2.3-2.6)	$<0.001$
LAD (cm)***	3.1 (2.9-3.3)	3.7 (3.3-4.0)	$<0.001$
LVEDD (cm)***	4.5 (4.3-4.7)	4.7 (4.4-5.0)	$<0.001$
IVST (cm)	0.9 (0.8-0.9)	0.9 (0.8-1.0)	0.094
RAD (cm)***	3.5 (3.3-3.8)	3.8 (3.5-4.2)	$<0.001$
RVEDD (cm)***	3.5 (3.2-3.6)	3.6 (3.4-3.9)	$<0.001$
LVEF (%)	65 (64-68)	65 (62-68)	0.536
MVE (m/s)	1.0 (0.8-1.1)	1.0 (0.9-1.2)	0.066
MVA (m/s)***	0.8 (0.6-0.9)	0.8 (0.8- 1.0)	$<0.001$
PH, n (%)***	5 (3.5)	22 (22.2)	$<0.001$
LV diastolic dysfunction, n (%)**	27 (19.0)	38 (38.4)	0.001
Mitral regurgitation, n (%)***	46 (33.4)	65 (65.7)	$<0.001$
Tricuspid regurgitation, n (%)*	79 (55.6)	69 (69.7)	0.027
Aortic regurgitation, n (%)***	9 (6.3)	28 (28.3)	$<0.001$

Continuous variables which are non-normally distributed were presented as median (interquartiles). Categorical variables were presented as number (proportions). Differences between groups were analyzed by using Mann-Whitney test and Chi-square test, respectively

\*  $P$ value < 0.05, \*\*  $P$ value < 0.01, \*\*\*  $P$ value < 0.001

**Table 3** Correlations of plasma CTGF level with thyroid related parameters

	Rs	$P$ -value
FT3*	0.129	0.046
FT4	0.091	0.161
TSH	-0.041	0.523
TPOAb	-0.031	0.630
TgAb	0.075	0.244
TRAb*	0.138	0.032
2-h RAIU	0.166	0.060
24-h RAIU	0.138	0.118
Thyroid mass	0.099	0.261

\*  $P$ value < 0.05

**Table 4** Correlations of plasma CTGF level with continuous parameters associated with cardiac

	Rs	P-value
Age	0.033	0.609
CK	0.086	0.183
CK-MB	0.135	0.108
LDH*	0.141	0.029
TNI*	0.291	0.018
BNP	0.070	0.454
AAOD	0.025	0.695
LAD*	0.146	0.023
LVEDD	0.050	0.442
IVST	-0.041	0.531
RAD*	0.132	0.041
RVEDD*	0.142	0.027
PAD	0.069	0.285
LVEF	0.012	0.856
MVE	0.043	0.504
MVA	0.119	0.066

\* P value < 0.05

**Table 5** The associations between plasma CTGF levels and HHD in the binary logistic regression analysis

		Quintiles of plasma CTGF levels							
		Quartile 1 (0~6.32 ng/ml)		Quartile 2 (6.32~7.91 ng/ml)		Quartile 3 (7.91~9.10 ng/ml)		Quartile 4 (>9.10 ng/ml)	
Modal	OR	OR (95%CI)	P-value	OR (95%CI)	P-value	OR (95%CI)	P-value	OR (95%CI)	P-value
Modal 1	1 (Ref)	1.427 (0.663-3.073)	0.364	2.529 (1.188-5.387)	0.016	2.529 (1.188-5.387)	0.016	2.529 (1.188-5.387)	0.016
Modal 2	1 (Ref)	1.759 (0.787-3.934)	0.169	2.496 (1.132-5.502)	0.023	2.976 (1.334-6.636)	0.008	2.976 (1.334-6.636)	0.008
Modal 3	1 (Ref)	1.739 (0.770-3.924)	0.183	2.551 (1.144-5.686)	0.022	3.034 (1.350-6.815)	0.007	3.034 (1.350-6.815)	0.007
Modal 4	1 (Ref)	1.732 (0.722-4.158)	0.219	1.995 (0.844-4.717)	0.116	2.540 (1.068-6.039)	0.035	2.540 (1.068-6.039)	0.035

Modal 1: unadjusted

Modal 2: age-and sex-adjusted

Modal 3: adjust for age, sex, duration of symptoms of hyperthyroidism and BMI

Modal 4: adjust for age, sex, duration of symptoms of hyperthyroidism, BMI, HR, SBP, DBP, FT4, TSH, TRAb, TPOAb

## Figures

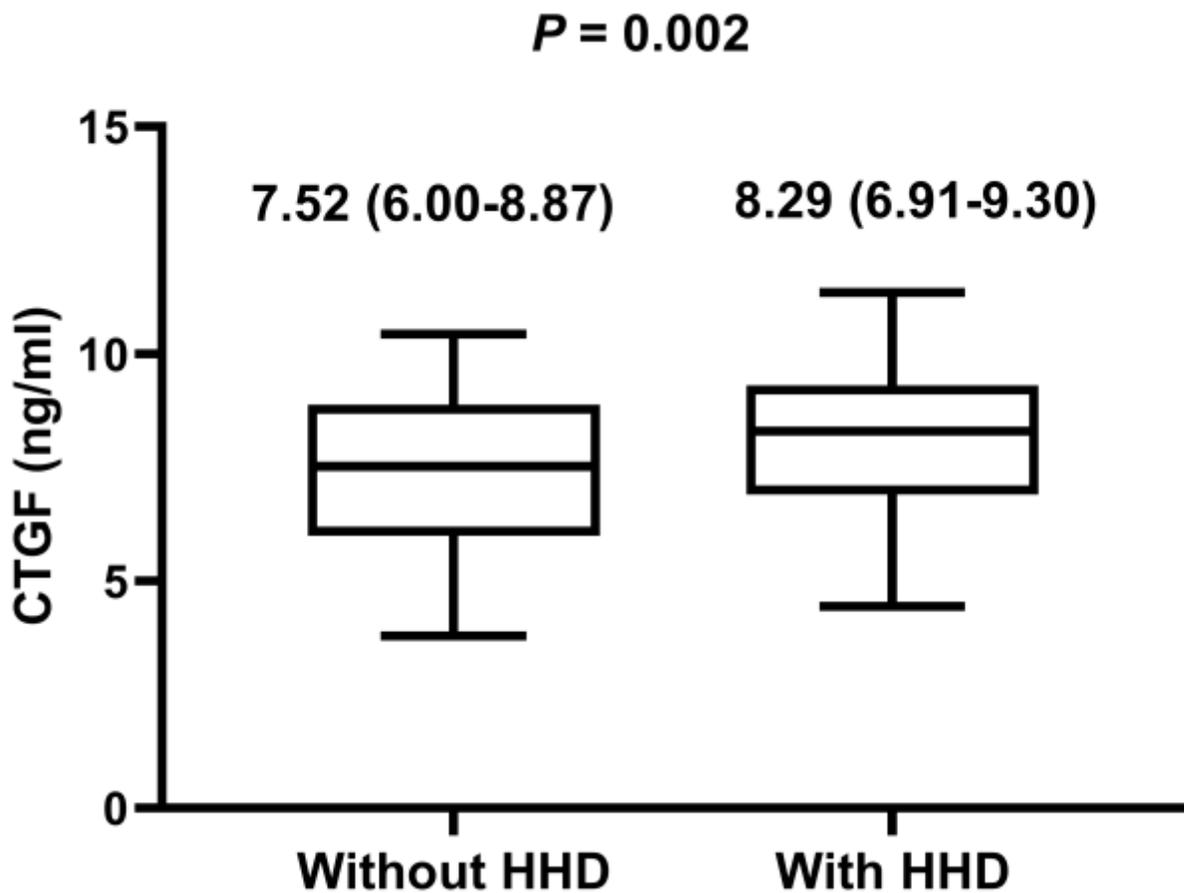


Figure 1

Comparison of circulating plasma CTGF levels between hyperthyroidism patients with or without hyperthyroid heart disease (HHD) The plasma CTGF levels are presented as median (interquartiles). Differences were analyzed by using the Mann-Whitney between two groups. Plasma CTGF levels in HHD are significantly higher than those without HHD ( $P = 0.002$ ).