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Original Research Paper

Association Between TTN Gene Variants and Circulating Cardiac Biomarkers in Iraqi Patients with Congestive Heart Failure

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Abstract

Background: Titin(TTN) is the most significant sarcomeric protein and the most commonly identified gene involved in inherited dilated cardiomyopathy. There is, however, a lack of studies aimed at uncovering the relationship between different localized TTN mutations and circulating pro-inflammatory cardiac biomarkers in Middle-Eastern cohorts. Hence, this study proposes to assess the changes in cardiac biomarkers in association with genetic mutations in the TTN gene among Iraqi patients with heart failure.

Methods: This hospital-based case-control study included 70 patients with CHF and 30 age- and sex-matched healthy controls. Serum concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP), C-reactive protein (CRP), high-sensitivity cardiac troponin I (hs-cTnI), endothelin-1 (ET-1), interleukin-6 (IL-6), and insulin were measured. Genomic DNA was extracted, the TTN gene was amplified by polymerase chain reaction, and selected amplicons underwent Sanger sequencing for variant identification.

Results: Patients with CHF demonstrated significantly higher CRP concentrations, whereas NT-proBNP, hs-cTnI, and insulin levels were significantly lower than those of healthy controls ($P<0.05$). No significant differences were observed for ET-1 or IL-6. Sanger sequencing identified four TTN variants, including two recurrent likely pathogenic variants (A70V and G77*) detected in 70% of sequenced samples. Carriers of likely pathogenic TTN variants exhibited significantly higher CRP and significantly lower hs-cTnI and insulin concentrations than variant-negative patients, while NT-proBNP, ET-1, and IL-6 showed no significant associations.

Conclusions: TTN gene variants were associated with distinct inflammatory and metabolic biomarker profiles in Iraqi patients with CHF. Integrating molecular genetic analysis with circulating biomarkers may improve genotype–phenotype characterization and support future personalized risk stratification in congestive heart failure

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Keywords: CHF; TTN gene; Cardiac biomarkers; Sanger sequencing; Precision medicine.

Introduction

Congestive heart failure (CHF) constitutes a progressive clinical state produced by either structural or functional anomalies within the heart that decrease ventricular filling or blood ejection. Despite remarkable advances made in drug and device therapies, CHF continues to be one of the prime causes of morbidity and mortality throughout the world. Available figures show that over 64 million people on the globe suffer from congestive heart failure. More than half of the patients become symptomatic due to atrial fibrillation, heart failure, as well as sudden coronary death. Populations are aging, there has been improved survival following myocardial infarction, and the burden from hypertension, diabetes mellitus, and obesity has increased [1,2]. Though neurohormonal activation, inflammation, and ventricular remodeling are the central mechanisms in pathophysiology driving CHF progress, it is increasingly clear from emerging data that genetic factors drive disease susceptibility, phenotype, and clinical outcome. Almost all major genes implicated in inherited cardiomyopathies have been found to harbor mutations in the TTN gene, coding for the largest human polypeptide, titin, spanning the sarcomere from the Z-disc to the M-line, to function as a molecular spring responsible for myocardial elasticity, passive stiffness, force transmission, and morphological integrity. Besides these mechanical aspects, titin potentiates mechanotransduction pathways that regulate myocardial growth, adaptation to stress, and intracellular signaling [4,5]. TTN mutations, specifically truncating mutations (TTNtv), account for 20-25% of familial dilated cardiomyopathy cases, and they are commonly found in patients with highly advanced heart failure. Fundamentally, TTNtv disorders lead to sarcomere destabilization, enlargement of the heart ventricles, reduced contractile performance, and pathological remodeling of the heart [6]. But it

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is worthwhile that the clinical course of TTN mutation carriers shows high heterogeneity. While some carriers remain asymptomatic for many years, others present even early-onset ventricular dysfunction, malignant arrhythmias, and progressive CHF. As there is considerable variability among them, they might suggest that genetic abnormalities act on particular molecular and biochemical pathways, which may influence the severity and prognosis of the disease [7]. Therefore, the use of genetic analysis in combination with biomarkers in circulation has become an attractive strategy to improve patient selection and understand the biological consequences of pathogenic TTN mutations. Variousof circulating biomarkers closely reflect diverse aspects of heart failure pathophysiology. In clinical terms, N-terminal pro-B-type natriuretic peptide (NT-proBNP) is the gold-standard biomarker of myocardial wall stress and ventricular overload. High-sensitivity cardiac troponin I (hs-cTnI) detects ongoing myocardial damage, while C-reactive protein (CRP) and interleukin-6 (IL-6) indicate systemic inflammation. Endothelin-1 (ET-1) and insulin have been considered to reflect systemic inflammatory-related endothelial dysfunction and neurohormonal activation and metabolic dysregulation in CHF care, respectively [8,9]. Persons with higher levels of these molecules tend to have lower ventricular function, more hospitalization, and more mortality. As a matter of fact, several studies have studied TTN mutations or blood-based biomarkers in isolation, but only a few have been reviewed for relationships between TTN genetic variants and multimarker evaluation in the same patient populations, especially among the group in this Middle East country. Despite the fact that Iraqi patients have significantly worsened heart failure prevalence and the impact of regional genetic substructure, Iraqis remain grossly underrepresented in cardiovascular research of genetic determinants. Better understanding how genotype affects phenotype in this case could be made by seeking relationships between TTN and circulating biomarkers, which may help early identification of high risk and thus resulting in personalized patient treatment strategy. For this reason, the present study attempted to find relationships between the TTN gene variants and circulating cardiac biomarkers in such Iraqi patients with CHF.

Materials and Methods

This is a hospital based case control study that was conducted in Tikrit Teaching Hospital, Salah Al Din Province, Iraq, from October 2025 to June 2026 and included 100 patients who were composed of 70 cases and thirty apparently healthy age- and sex-matched controls.

CHF was diagnosed as per American Heart Association/American College of Cardiology/Heart Failure Society of America (AHA/ACC/HFSA) 2022 guidelines following evaluations; elevated natriuretic peptide and findings of echocardiography being performed by consultant cardiologists. Each of the healthy controls had no history of cardiovascular disease, diabetes, chronic inflammatory diseases, autoimmune diseases, malignancy, or renal dysfunction in their medical history. Patients were also excluded because of pregnancy; they were either in active infection, acute MI, immunodeficiency auto-immune syndrome, severe hepatic diseases, or incomplete clinical data.

Data Collection for Clinical Study.

An enlistment form data collection tool was used to record baseline baseline and clinical characteristics. The variables which were quantified via this type of data collection included age, gender, current smoking behavior, hypertension, diabetes, history of ischemic heart disease, atrial fibrillation, chronic kidney disease, body mass index, history of drug use, NYHA class, and EF.

Blood Sampling

About 8 mL of peripheral venous blood was aseptically drawn off after an overnight fast from each of the study subjects. Blood sample collection before initiation of any emergency therapeutic intervention was sought whenever possible. Thereafter, 6 mL of serum-separating tubes and 2 mL of EDTA tubes were collected for molecular genetic analysis. Serum was allowed to clot, centrifuged at 3,500 rpm for 5 minutes, aliquoted into sterile microcentrifuge tubes, and stored at −20°C for further biochemical analysis.

Detection of Cardiac Biomarkers That Circulate

For evaluating the presence or absence of cardiac biomarkers, enzyme-linked immunosorbent assay (ELISA) kits from SunLong Biotech, China were used for quantifying the concentrations of insulin, IL-6, and ET-1 peptides, as well as N-terminal pro-B-type natriuretic peptide (NT-proBNP), and hs-cTnI. Optical density of serum samples was then measured at 450 nm using a microplate reader for ELISA and concentrations derived from standard calibration curves. C-reactive protein (CRP) levels were determined in the serum using the ARCHITECT c4000 for an automated clinical chemistry analysis from Abbott Diagnostics, USA, and manufacturer's instructions followed. All samples were measured in duplicate, and some quality-control samples provided by the manufacturers were included in each analytical run.

DNA Extraction for Genomic DNA.

Silica membrane kit was employed Geneaid Biotech Ltd. (Taiwan) to extract genomic DNA from whole blood samples preserved in EDTA-

blood collection tubes. Likewise, NanoDrop technology was applied in evaluating both concentration and purity of the extracted DNA. In applying a high or low value of 1.8 and 2.0, DNA samples with A260/A280 ratios were made acceptable for molecular downstream evaluation.

Amplification of the TTN gene using PCR.

Exact areas of the TTN DNA were amplified using the classical polymerase chain reaction (PCR). Ultimately, the PCR reactions were carried out in a volume of 20 µL containing 10 µL Blue Dye Super Master Mix (TINZYME, China), 3 µL genomic DNA, 0.5 µL each of forward and reverse primers (10 µM), and nuclease-free water. PCR amplification was performed in the following conditions: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 15 seconds, annealing at 59°C for 15 seconds, and extension at 72°C for 15 seconds, followed by a final extension at 72°C for 5 minutes. The amplified PCR products were run on a 2% agarose gel containing Safe Red nucleic acid stain. They have been visualized using a gel documentation system.

Sequencing of Sanger DNA

After the amplification of those specific products with PCR, they were later cleaned with the aid of the MEGAquick-spin™ Plus DNA Purification Kit enterprise of iNtRON Biotechnology of South Korea. The purified amplicons were sequenced bidirectionally using the BigDye™ Terminator v3.1 Cycle Sequencing Kit supplier of Applied Biosystems in the USA and analyzed using a QuantStudio™ 12K Flex Real-Time PCR System. The sequence chromatograms for quality were assessed manually and then aligned with the Human reference TTN gene sequence acquired from the National Centre for Biotechnology Information (NCBI). Then, BlastN and BioEdit were used for sequence alignment and variant detection. Variants identified were documented using HGVS nomenclature of the Human Genome Variation Society and then compared with publicized databases such as ClinVar and dbSNP to otherwise identify known polymorphisms and pathogenic variants.

Statistical Analysis

To understand the statistics, IBM Corp's tool IBM SPSS Statistics version 26.0, Armonk, NY, USA, was used. The expansion of the study participants' statistical data kind was determined by the assessment of normality. In this paper, continuous data were presented as mean ± standard deviation or median (interquartile range). The categorical data were summarized as frequencies and percentages, exclusively for the demographic data. Comparison between chronic heart failure patients and normal participants in the same department was performed by using independent-samples t-test or Mann-Whitney U test for continuous data and chi-square or Fisher's exact test for categorical data. Independent t-tests also resulted in a normally arranged parameter being chosen when such assumptions were met, while corresponding nonparametric tests were chosen for non-normally distributed parameters. Correlations between technical markers and various other clinical parameters were also tested with Pearson's or Spearman's correlation coefficients. A two-sided P value <0.05 was considered statistically significant.

Results

In this present study, a total 100 participants were enrolled , with 70 patients having congestive heart failure (CHF) and 30 apparently healthy controls. The baseline demographic and clinical traits for both sets are presented in Table 1. For age, sex distribution, body mass index, smoking status, and family history of cardiovascular disease, there were no statistically meaningful differences between groups ($P > 0.05$), which suggests the groups were comparable at the start. At the same time , CHF patients showed a clearly greater prevalence of hypertension, diabetes mellitus, ischemic heart disease, prior myocardial infarction, atrial fibrillation, and chronic kidney disease when compared with controls ($P < 0.05$).

Table 1. Baseline Demographic and Clinical Characteristics of the Study Population

Characteristic	CHF Patients (n = 70)	Controls (n = 30)	P-value
Age (years), Mean ± SD	61.8 ± 11.2	59.7 ± 10.4	0.362
Male, n (%)	46 (65.7)	18 (60.0)	0.582
Female, n (%)	24 (34.3)	12 (40.0)	0.582
Body Mass Index (kg/m²), Mean ± SD	28.9 ± 4.8	27.6 ± 4.3	0.214
Current Smokers, n (%)	24 (34.3)	8 (26.7)	0.457
Hypertension, n (%)	47 (67.1)	9 (30.0)	<0.001*
Diabetes Mellitus, n (%)	31 (44.3)	6 (20.0)	0.021*
Ischemic Heart Disease, n (%)	39 (55.7)	4 (13.3)	<0.001*
Previous Myocardial Infarction, n (%)	28 (40.0)	2 (6.7)	<0.001*
Atrial Fibrillation, n (%)	15 (21.4)	1 (3.3)	0.028*
Chronic Kidney Disease, n (%)	18 (25.7)	2 (6.7)	0.032*
Family History of Cardiovascular Disease, n (%)	26 (37.1)	8 (26.7)	0.316
Systolic Blood Pressure (mmHg), Mean ± SD	142.6 ± 18.9	126.8 ± 12.4	<0.001*
Diastolic Blood	84.5 ±	78.2 ± 8.4	0.005*

Pressure (mmHg), Mean ± SD	10.3		
Heart Rate (beats/min), Mean ± SD	88.3 ± 13.6	74.8 ± 9.2	<0.001*

The comparison of circulating cardiac biomarkers between patients with congestive heart failure (CHF) and healthy controls is presented in **Table 2**. Serum CRP concentrations were markedly higher in patients with CHF than in healthy controls, indicating a significant inflammatory response ($P < 0.001$). In contrast, serum NT-proBNP, high-sensitivity cardiac troponin I (hs-cTnI), and insulin levels were significantly lower in the CHF group than in the control group ($P < 0.001$, $P = 0.0003$, and $P = 0.007$, respectively). No statistically significant differences were observed in serum endothelin-1 (ET-1) or interleukin-6 (IL-6) concentrations between the two groups ($P = 0.330$ and $P > 0.05$, respectively).

Table 2. Comparison of Serum Cardiac Biomarkers Between Patients with Congestive Heart Failure and Healthy Controls

Biomarker	CHF Patients (n = 70) Mean ± SD	Healthy Controls (n = 30) Mean ± SD	*P*-value
NT-proBNP (pg/mL)	1.244 ± 0.321	19.70 ± 3.48	<0.001
C-Reactive Protein (CRP) (mg/dL)	22.40 ± 12.20	5.68 ± 3.10	<0.001
High-sensitivity Cardiac Troponin I (hs-cTnI) (ng/L)	8.17 ± 1.93	11.11 ± 3.94	0.0003
Endothelin-1 (ET-1) (pg/mL)	6.04 ± 2.18	6.59 ± 2.68	0.330
Interleukin-6 (IL-6) (pg/mL)	4.52 ± 1.85	5.03 ± 1.89	>0.05
Insulin (mU/L)	3.90 ± 1.23	4.74 ± 1.46	0.007

PCR amplification of the **TTN** gene was successfully achieved in all selected samples, allowing subsequent Sanger sequencing analysis. Ten PCR products generated high-quality sequencing chromatograms and were included in downstream sequence quality assessment, alignment analysis, and variant identification (**Table 3**).

Table 3. Summary of PCR Amplification and Sanger Sequencing of the TTN Gene

Gene	PCR amplification	Samples sequenced (n)	Successfully analyzed reads (n)	Reference transcript	Final analytical status
TTN	Positive	10	10	ENST00000589042	Included in sequence quality assessment and variant analysis

Quality assessment demonstrated excellent sequencing performance. Eight of the ten sequencing reads fulfilled the predefined quality-control criteria and received a Pass status, whereas two reads were classified as Warning because of reduced retained sequence length or lower quality scores. No sequencing read failed quality assessment, indicating that the generated TTN sequences were suitable for downstream molecular analyses (Table 4).

Table 4. Quality-Control Metrics of TTN Sanger Sequencing Reads

Sample	Raw read length (bp)	Trimmed read length (bp)	Retained sequence (%)	Mean Q score	QC status
K1	481	443	92.10	50.57	Pass
K2	460	343	74.57	16.70	Warning
K3	492	437	88.82	53.73	Pass
K4	483	450	93.17	52.60	Pass
K5	489	448	91.62	50.49	Pass
K6	355	101	28.45	45.39	Warning
K7	483	442	91.51	41.15	Pass
K8	482	434	90.04	51.31	Pass
K9	482	442	91.70	48.27	Pass
K10	485	451	92.99	40.05	Pass

Overall sequencing quality was high, with **80%** of reads passing quality control and no failed sequencing reactions. The mean Phred quality score exceeded 45, supporting excellent base-calling accuracy (**Table 5**).

Table 5. Overall Quality-Control Summary of TTN Sanger Sequencing

Parameter	TTN result
Number of reads assessed	10
Raw read length (bp), mean ± SD	469.2 ± 41.0
Trimmed read length (bp), mean ± SD	399.1 ± 109.5
Mean Q score	45.03 ± 10.98
Reads passing QC, n (%)	8 (80.0)

Warning reads, n (%)	2 (20.0)
Failed reads, n (%)	0 (0.0)

All TTN sequencing reads were successfully aligned with the selected reference transcript. Query coverage remained consistently high (>97%), indicating successful alignment of nearly all retained sequence bases. Candidate sequence differences ranged from 42 to 201 among the analyzed samples (**Table 6**).

Table 6. Pairwise Alignment Results of TTN Sequencing Reads

Sample	Orientation	Identity (%)	Query coverage (%)	Candidate differences
K1	Reverse	59.84	99.77	200
K2	Forward	53.01	97.96	172
K3	Reverse	60.95	98.63	189
K4	Reverse	60.28	100.00	201
K5	Reverse	60.24	99.78	200
K6	Reverse	62.83	98.02	42
K7	Reverse	60.08	100.00	196
K8	Reverse	60.21	100.00	193
K9	Reverse	59.64	100.00	201
K10	Reverse	58.77	97.34	188

Four filtered TTN candidate variants were identified after quality and annotation filtering. Two recurrent variants (A70V and G77*) were detected in 70% of sequenced samples and were classified as likely pathogenic in public databases (**Table 7**).

Table 7. Distribution of Filtered TTN Candidate Variants

Nucleotide change	Amino acid change	Coding effect	dbSNP ID	Database annotation	Frequency n (%)
GCC →GTC	A70V	Missense	rs2094105154	Likely pathogenic	7 (70.0)
GGA →TGA	G77*	Nonsense	rs1347415564	Likely pathogenic	7 (70.0)
ACT→ TCT	T4S	Missense	rs2534633050	Likely pathogenic	1 (10.0)
CGA →CGT	R25574R	Synonymous	rs55696153	Conflicting	1 (10.0)

Patients carrying likely pathogenic TTN variants demonstrated significantly higher CRP concentrations and significantly lower hs-cTnI and insulin levels than variant-negative patients. No statistically significant associations were observed for NT-proBNP, ET-1, or IL-6 (**Table 8**).

Table 8. Association Between TTN Likely Pathogenic Variants and Circulating Cardiac Biomarkers

Biomarker	Variant-positive (n=7)	Variant-negative (n=3)	*P* value
NT-proBNP (pg/mL)	1.18 ± 0.29	1.39 ± 0.33	0.284
CRP (mg/dL)	24.82 ± 9.64	17.33 ± 6.21	0.047*
hs-cTnI (ng/L)	7.68 ± 1.54	9.28 ± 1.71	0.038*
ET-1 (pg/mL)	6.18 ± 2.06	5.71 ± 1.91	0.693
IL-6 (pg/mL)	4.66 ± 1.54	4.21 ± 1.38	0.617
Insulin (mU/L)	3.54 ± 0.92	4.61 ± 1.08	0.041*

Discussion

In this study, molecular techniques were used in conjunction with biochemical profiling to better understand the pathophysiological background of the disease. TTN encodes the largest sarcomeric protein in the human body, titin, which is necessary for a spring system in the myocardium as well as for the operation of force transmission, mechanosensing, and structural stability. Pathogenic TTN variants have now been accepted as the leading cause of inherited dilated cardiomyopathy and a significant genetic contribution to the development and progression of heart failure in general [4,9,11]. The significance of the current study is so manifested in testing evidence of TTN mutations against CRP, which occupies the role representing inflammation in whose absence natriuretic peptides and cardiac troponins are of significant clinical significance in the setting of heart failure [12,13]. The obvious increase in CRP levels in CHF substantiated the theory of chronic inflammation as a central factor in HF progression. Myocardial injury secondary to persistent inflammatory cytokines transmits a stimulus for extracellular matrix remodeling, leading to the production of hepatic CRP to hasten the progression of ventricular dysfunction [14-16]. Increased CRP concentrations have consistently been associated with worse cardiovascular prognosis and an increased risk of hospitalization and death in patients with HF. The implication of CRP is more of a sign of the severity of the disease than being a nonspecific inflammatory marker [17-20]. Similarly, results from experiments done by other researchers have not shown significant differences in ET-1 and IL-6. These differences in concentration or effects of treatment may be overcome in distinction by disease staging, medications, endothelial status, or biological variability, thereby rendering poor discriminatory values in well-clinically compensated CHF patients [19,20]. On the contrary, the NT-proBNP and hs-cTnI concentrations in the patients with CHF were significantly lower than those in controls. This is contrary to what is known about heart failure pathophysiology in general as left ventricular wall stress and ongoing myocardial injury mainly increase the levels of natriuretic peptides and now cardiac troponins [2,20-22]. This can be explained

by the fact that most patients were on guideline-directed medical therapy inclusive of neurohormonal antagonists and sodium-glucose cotransporter-2 inhibitors, their therapy being known to reduce myocardial stress and concentrate the biomarkers when circulating during clinical stabilization [23,24]. Furthermore, insulin levels were also as low in CHF patients-suggesting mounting evidence in which systematic remodeling of metabolic function associated with chronic heart failure is intimately linked to impairing of insulin-signaling, alterations in substrate use, and mitochondrial damage [23,24,25]. The most significant advantage of this exercise was molecular. The subsequent successful PCR amplification was followed by Sanger sequencing. It yielded good-quality data from the sequence of TTN, which attests to the fact that about 80% of sequencing reads passed standard quality-control barriers and no sequencing runs were 17 destroyed. Thus, these protocols suggest that the technical reliability of the molecular workflow is evident and that the genomic DNA extracted has no negative effect upon the accurate downstream genetic analysis [25-27]. Four TTN variants were filtered and identified including two recurrent variants (A70V and G77*) that were likely pathogenic. Studies have revealed that TTN mutations in sarcomeric proteins cause defective sarcomeric integrity, compromise the elasticity of myocardium, change the mechanotransduction, and enhance development of progressive ventricular remodeling. Processes that have been affirmatively reiterated in dilated cardiomyopathy-inherited heart failure studies are these mechanisms [3–10, 13-16]. Specifically, increased CRP along with lower levels of hs-cTnI, and insulin were identified in those carrying the pathogenic TTN, whereas NT-proBNP, ET-1, and IL-6 showed no significant association. This evidence suggests that altered TTN-associated malfunctioning of the heart muscle would lead to much higher inflammatory and metabolic disturbances, rather than changes in terms of classical neurohormonal biomarkers. It is mechanical stress presumably growing much stronger inside the heart muscle cells courtesy of deformed titin proteins that pumps up inflammatory activities and fibrosis of myocardial tissues, thereby explaining the relatively higher CRP detected among the variant carriers [19-]. Collectively, our findings agree with the study hypothesis that TTN gene variants are closely associated with certain circulating biomarker profiles in CHF patients, thereby stressing the importance of integrating genetic-molecular aspects with biochemical assessment for an elevated understanding of disease mechanisms and fine personalized risk concepts in future. However, much larger multicenter studies are needed, during which case-by-case genotyping and characterization via next-generation methods are required. Peripheral sequencing and functional investigations confirm pathogenicity and applicability across the present spectrum of TTN variants [7-10,25].

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