Journal of King Saud University – Science 35 (2023) 102440

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Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Association of interleukin-18 promoter polymorphism with comorbid conditions of cardiovascular disease

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ARTICLE INFO

Article history:
Received 13 September 2022
Revised 5 November 2022
Accepted 7 November 2022
Available online 11 November 2022

Keywords:
Cardiovascular disease
Comorbidity
Diabetes
Gene polymorphism
Interleukin-18

ABSTRACT

Objectives: Cardiovascular disease (CVD) is one of the most clinically relevant pathologies that remains the largest single contributor to global mortality. It is often associated with more than one comorbid condition, which obscures its clinical outcome. The current study aimed to evaluate the possible association of interleukin-18 (IL-18) promoter polymorphism with comorbid conditions of CVD.
Methods: We used case-control comparison of specific genotypes of three clinically relevant IL-18 polymorphism hotspots, viz. −656 T/G (rs1946519), −607C/A (rs1946518), and −137 G/C (rs187238) with commonly associated comorbid conditions of CVD such as diabetes, hypertension, and dyslipidemia. For this study, whole blood of CVD patients and healthy control subjects were collected in a citrate coated/plain tube. The routine biochemical parameters were estimated in each sample, and DNA samples were extracted for PCR amplification for further sequencing of targeted amplicons using Sanger method.
Results: The studied biochemical parameters showed a significant increase in CVD patients compared with control individuals. Fasting glucose and glycosylated hemoglobin (HBA1C) showed an increase from 4.82 to 8.6 ($p < 0.05$) and 4.33 to 8.2 ($p < 0.05$), respectively. The results showed a statistically significant association with CVD-diabetes and CVD-hypertension group with GG, GC, and CC genotype at IL-18 gene locus, rs187238. On the other hand, the CVD-dyslipidemia group showed a positive association with allele distribution at the same hotspot. In addition, the GG, GT, and TT genotype and G and T allele distribution at rs1946519 locus showed statistically significant association with CVD-diabetes, CVD-hypertension, and CVD-dyslipidemia p compared with control subjects. We also observed a statistically significant association of dyslipidemia with three genotypic combinations viz. (rs1946518 AA, rs1946519 GG, rs187238 GG); (rs1946518 AA, rs1946519 GT, rs187238 GG), and (rs1946518 AA, rs1946519 TT, rs187238 GG).
Conclusions: Based on our study, we conclude that IL-18 loci, rs1946519 has a significant association with each studied comorbid condition and can be considered a prognostic marker of CVD and comorbidities. Our results are anticipated to be utilized to launch a significant pharmaco-genomic investigation that could identify patients with comorbidities who are more likely to develop CVD.

1. Introduction

Cardiovascular disease (CVD) is a clinically relevant pathologies driven by highly heterogeneous and complex biological pathways (Nwadiugwu 2021, Tabrez et al., 2022). It remains the largest single contributor to global mortality, and approximately 19 million global fatalities was attributed to CVD in 2020, which amounted to an increase of 18.7 % from 2010 (Tsao et al., 2022). More than 45 % of all deaths in the Gulf Council Countries (GCC), including Saudi Arabia, are attributed to CVD, a proportion that is significantly higher than that of the world and developed nations (Alhabib et al., 2020).

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2022.102440

Comorbidity is the simultaneous occurrence of one or more chronic conditions with an index disease leading to complicated diseased conditions with greater mortality (Buddeke et al., 2019). An increasing amount of scientific evidence indicates that CVD is typically associated with more than one comorbid condition, such as type 2 diabetes (T2DM), hypertension, dyslipidemia etc. Lately, comorbidity has been considered a crucial factor in CVD management. The incidence of typical comorbid conditions of CVD, including T2DM, hypertension, and dyslipidemia, are also observed at a higher rate in the GCC region which is expected to result in the increased burden of this devastating disease (Aljefree and Ahmed 2015, Alhabib et al., 2020).

Early identification of risk factors, clinical manifestations, and their predictive ability have a seminal role in preventing diseases and comorbidities. According to the Interheart/stroke studies, hypertension, diabetes, and dyslipidemia can complicate CVD to terminal symptoms, such as myocardial infarction (MI) and strokes (Aljefree and Ahmed 2015). Lately, our group has highlighted different risk factors, leading to CVD progression and suggested some prognostic markers of CVD in the Saudi population (Firoz et al., 2015, Jabir et al., 2015, Jabir et al., 2016, Tabrez et al., 2017b).

The chronic inflammatory process arbitrated by a sequence of molecular events producing several pro-inflammatory cytokines has been considered the dominant cause of CVD (Jabir and Tabrez 2016). Formation and accumulation of inflammatory atheromatous plaques within the walls of the coronary arteries trigger the manifestation of different CVD symptoms. The complexity of specific inflammatory mechanisms and destabilization of coronary plaques is well documented in the scientific literature (Nwadiugwu 2021). Atheromatous plaques are more prone to rupture, leading to eventual thrombotic manifestations in the coronary arteries (Libby et al., 2018). The limitation of diagnostic and therapeutic strategies to alleviate the burden of CVD complications demands more efforts from the scientific community to get successful outcomes..

The initiation and progression of atherosclerotic lesions, starting with endothelial dysfunction and ending with plaque development, instability, and rupture (Nguyen et al., 2019). Atherosclerotic lesions release a number of competent immune cells, especially proinflammatory cytokines to participate in the different pathological events and contribute to fatal outcomes in the form of MI (Jabir and Tabrez 2016). Interleukins are expressed by all cell types and serve as promoters/predictors of CVD pathological events and their comorbidities (Cavalcante et al., 2020, Gager et al., 2020). Inflammatory responses also contribute significantly in the onset and advancement of T2DM, hypertension, and dyslipidemia (Nwadiugwu 2021). Hindering the inflammatory pathway by timely suppressing the specific inflammatory mediators can contain injury and provide optimal infarct healing (Jabir and Tabrez 2016, Anzai et al., 2022). In the last few years, our research group has reported the possible role of several cytokines and chemokines in Saudi CVD patients (Firoz et al., 2015, Jabir et al., 2016, Jabir et al., 2017a, Jabir et al., 2017b, Tabrez et al., 2017a, Tabrez et al. 2017b, Tabrez et al., 2021, Tabrez et al., 2022).

Genomics is a potent tool to discover novel biomarkers and biochemical pathways to enhance early determination of disease and might provide pathological indulgence of diseases beyond traditional methods (Zhang et al., 2020). The genomic study on different inflammatory pathways holds several advantages to screen and distinguish CVD causation, progression, and prognosis (Alfaddagh et al., 2020). The last decade has witnessed extensive advance in understanding heart diseases, highlighting the role of several interleukin associated genetic modulations in CVD (Lian et al., 2019, Zhang et al., 2020). Genetic variants of specific genes or DNA sequence at different loci have also been reported to be linked with the susceptibility of CVD (Lian et al., 2019, Cavalcante et al., 2020). Despite of the disparity in findings, several studies reported the association of pro-inflammatory interleukin gene polymorphisms with CVD susceptibility, particularly IL-1, IL-4, IL-6, IL-8, and IL-18 in different ethnic populations (Zhang et al., 2019, Rechciński et al., 2021, Tabrez et al., 2021, Tabrez et al., 2022). DNA sequence variants have the unique potential to predict causal biologic mechanisms, novel pathophysiological pathways, and specific molecular interactions across disease-relevant tissues.

Identifying of genetic polymorphisms in inflammatory cytokines could enable early prediction of CVD and provide sensible management and progression (Tabrez et al., 2021, Tabrez et al., 2022). In addition, the identification of genetic variants also uncovers the potential opportunity to develop novel molecular targets for blocking atherosclerosis at an early stage.

In the recent past, our research group has reported the influence of various interleukin promoter polymorphisms with CVD and its associated conditions. We aim to explore the interleukin-18 promoter polymorphism further and identify its possible association with CVD comorbidities. For this purpose, the three polymorphic hotspots of interleukin-18 gene promoter −656 T/G (rs1946519), −607C/A (rs1946518) and −137 G/C (rs187238) were analyzed. We expect that our research could provide new genotypic perspectives on the influence of comorbidities on CVD progression.

2. Materials and methods

The study included 76 participants, both male and female, who have been admitted to the KAU hospital in Jeddah with angiographically confirmed CVD. A control group of 50 healthy individuals were used for comparison purposes. Through the use of vascular, echocardiographic, radiographic, and biochemical criteria, the clinical symptoms of CVD were verified. The standard definitions of diabetes, hypertension, and dyslipidemia were also considered. Exclusion criteria included patients who were hesitant to participate, had major uncontrolled conditions, had any form of cancer or inflammatory disorders, were taking anti-inflammatory medications, or had an infection during the preceding two months. Statin, aspirin, metformin, clopidogrel, and antihypertensive drugs (amlodipine, bisoprolol, captopril etc) were taken by the majority of CVD patients. Moreover, none of the indicated medicines were regularly used by healthy controls. The current study received ethical approval (Reference No. 84–16) from the institutional committee, Jeddah, KSA. All those included in the study provided their written, fully informed consent. The Declaration of Helsinki served as the foundation for this study's design.

2.1. Sample collection

Each participant in this study provided 5 ml of peripheral blood, which phlebotomist withdrew into simple vials containing EDTA. For a subsequent biochemical examination, serum separation was performed by centrifuging blood samples at 2000g for 5 min. Using a solution-based DNA extraction kit, 200 μL of whole blood was used to extract the genomic DNA (GeneJET, Thermo Fisher Scientific, USA). The isolated DNA's quality, amount, and integrity were validated by spectrophotometric technique on a Nano drop. Until further examination, all extracted samples were kept at −80 °C.

2.2. Biochemical parameter estimation

Utilizing the Selectra ProM clinical chemistry analyzer, measurements of fasting blood glucose and lipid profile were made (ELITech, Sees, France). According to the manufacturer's instructions, the Dimension VistaTM System (Siemens Healthcare Diag-

nostics, Camberley, UK) was used to measure HbA1c. All samples were measured in triplicates, and the manufacturer's protocol was properly followed.

2.3. IL-18 promoter polymorphism genotyping

The PCR amplification was used to evaluate the IL-18 promoter polymorphisms at three hotspots [-137 G/C (rs187238), −607C/A (rs1946518), and −656 T/G (rs1946519)]. Sanger sequencing of the targeted gene was then performed as per the method described earlier (Jabir et al., 2016).

2.4. Statistical analysis

Version 25.0 of IBM SPSS Statistics for Windows 10 was used for all data analysis (IBM, USA). Based on the results of the preceding investigation, the patients were grouped as suggested by Cavalcante et al. 2020 (Cavalcante et al., 2020). For categorical data, chi-square tests were employed, and for continuous variables, one-way analysis of variance (ANOVA) was used. A percentage or mean ± standard deviation was used to express the data. $p < 0.05$ were deemed to be significant differences.

3. Results

The purpose of this study was to identify the possible association of IL-18 promoter polymorphisms at three different hotspots [-137 G/C (rs187238), −607C/A (rs1946518), and −656 T/G (rs1946519)] with well-known CVD comorbidities such as, diabetes, hypertension, and dyslipidemia. The study subjects' grouping was based on a specific genotype's presence. The rs187238 hotspot has a homozygous GG genotype, heterozygous GC genotype, and homozygous CC genotype that were used for comparative analysis with comorbid conditions. Whereas rs1946518 hotspot has homozygous CC genotype, heterozygous CA genotype, and homozygous AA genotype. Similarly, rs1946519 hotspot has a homozygous GG genotype, heterozygous GT genotype and homozygous TT genotype. The difference in total number of samples in each group was due to the unclear signals in DNA sequencing results and were omitted during analysis.

3.1. Baseline characteristics of the studied subjects

Table 1 presents the baseline characteristics of every individual who participated in this study. The mean age of the healthy controls and CVD patients were 50.26 ± 4.32 and 60.4 ± 8.5, respectively. Out of these, 72 % of the control individuals and 74.36 % of CVD patients were males. Among the CVD patients 55.13 % were diabetic, 76.92 % were hypertensive, and 80.77 % were dyslipidemic (comorbid conditions). The biochemical markers of diabetes viz. fasting blood sugar and HbA1C were found to be 4.82 vs 4.33 in controls and 8.6 vs 8.2 in CVD patients. On the other hand, the dyslipidemia markers such as total cholesterol, triglycerides, and HDL were found to be 4.42; 1.66; 1.15; 2.48 in controls, and 4.2; 1.7; 1.1; 2.8 in CVD patients, respectively.

Table 1
Clinical characteristics and biochemical profile of control and CVD subjects.

	Control (n = 50)	CVD patients (n = 76)
Age (Years)	50.26 ± 4.32	60.4 ± 8.5
Males (%)	36 (72 %)	58 (74.36 %)
Diabetes mellitus (%)	5 (9 %)	43 (55.13 %)
Hypertension (%)	7 (14 %)	60 (76.92 %)
Dyslipidaemia (%)	9 (17 %)	63 (80.77 %)
Fasting blood sugar (mmol/L)	4.82 ± 0.71	8.6 ± 3.4
HBA1C (%)	4.33 ± 0.54	8.2 ± 2.0
Total cholesterol (mmol/L)	4.42 ± 0.85	4.2 ± 1.3
Triglyceride (mmol/L)	1.66 ± 0.41	1.7 ± 1.02
HDL (mmol/L)	1.15 ± 0.22	1.1 ± 0.3
LDL (mmol/L)	2.48 ± 0.6	2.8 ± 1.0

Data given as mean ± SD or n (%); HBA1C, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

3.2. Genotypes and allele frequencies of IL18 −137 G/C (rs187238) polymorphism in CVD patients and their association with comorbid conditions

We compared the genotypes and allele frequencies of IL18 −137 G/C (rs187238) polymorphism of healthy controls and CVD with comorbidities viz. diabetes, hypertension, and dyslipidemia. The polymorphism in IL-18 gene at −137 G/C (rs187238) hotspot in CVD patients showed 65.3 % GG genotype, 22.5 % GC genotype and 6 % CC genotype with 76.5 % and 23.5 % G and C alleles in control subjects. The association of CVD with diabetic comorbidity showed 70.6 % GG, 20.6 % GC, and 8.8 % CC genotypes which was found to be statistically significant ($p < 0.05$). On the other hand, the allele frequencies of CVD with diabetic comorbidity were found to be 80.9 % G and 19.1 % C compared with 76.5 % G and 23.5 % C in control individuals. Similarly, the association of CVD with hypertension showed the genotypic pattern as 63.5 % GG, 25 % GC, and 11.5 % CC which was found to be highly significant ($p < 0.01$) compared with control genotypes. The allele frequencies of CVD with hypertension were 76.0 % vs 76.5 % G and 24.0 % vs 23.5 % C compared with controls. The association of CVD with dyslipidemia showed the genotypic pattern as 67.3 % of GG, 21.8 % of GC, and 10.9 % of CC genotype which were found to be statistically non-significant compared with controls. However, the allele frequencies of CVD with dyslipidemia showed highly significant ($p < 0.01$) association compared with controls. The G and C allele frequencies were found to be 78.2 % and 21.8 %, respectively in CVD patients with dyslipidemia conditions (Table 2).

3.3. Genotypes and allele frequencies of IL18 −607C/A (rs1946518) polymorphism in CVD patients and their association with comorbid conditions

The polymorphism site at −607C/A (rs1946518) hotspot in IL-18 gene showed 79.6 % CC genotype, 2 % CA genotype, and 9 % AA genotype with 80.6 % and 19.4 % of C and A alleles, respectively in control subjects. The CVD with diabetic comorbidity was significantly associated ($p < 0.05$) and showed 86.1 % of CC and 13.9 % AA genotype with C and A allelic frequency of 86.1 % and 13.9 %, respectively. The genotype and allele frequency with hypertension and dyslipidemia were not statistically significant with patients with CVD compared to controls. The CVD with hypertension showed 84.6 % and 15.4 % CC and AA genotype with an allelic frequency of 84.6 % and 15.4 % C and A, respectively. Similarly, CVD with dyslipidemia patients showed 90.7 % CC, and 9.3 % AA genotype with an allele frequency of 90.7 % and 9.3 % of C and A, respectively. All the patient groups did not express the heterozygous CA genotype (Table 3).

3.4. Genotypes and allele frequencies of IL18 −656 T/G (rs1946519) polymorphism in CVD patients and their association with comorbid conditions

Controls and CVD subjects' genotypic and allelic frequencies of IL18 −656 T/G (rs1946519) hotspots showed a highly significant association with studied comorbidities (Table 4). The rs1946519 hotspot showed 34.1 % GG genotype, 41.5 % GT genotype, and

Table 2
Distribution of genotype and allele frequencies of IL 18–137 G/C (rs187238) polymorphism in CVD comorbid conditions and control subjects.

IL18 −137 G/C (rs187238)				
Genotype frequency				
	Control individuals (%) (n = 49)	**CVD + Diabetes counts (%) (n = 34)**	**CVD + Hypertension counts (%) (n = 52)**	**CVD + Dyslipidemia counts (%) (n = 55)**
GG	32 (65.3)	24 (70.6)	33 (63.5)	37 (67.3)
GC	11 (22.5)	7 (20.6)	13 (25.0)	12 (21.8)
CC	6 (12.2)	3 (8.8)	6 (11.5)	6 (10.9)
P-value		**< 0.05**	**< 0.01**	NS
Allele frequencies				
G	75 (76.5)	55 (80.9)	79 (76.0)	86 (78.2)
C	23 (23.5)	13 (19.1)	25 (24.0)	24 (21.8)
P-value		NS	NS	**< 0.01**

Data are expressed as absolute numbers and percentages in parentheses. GG: wild-type, CC: homozygote; GC: heterozygote. The chi-square test was used for comparative analysis, and p-value < 0.05 was considered a significant criterion. NS: non-significant.

Table 3
Distribution of genotype and allele frequencies of IL 18–607C/A (rs1946518) polymorphism in CVD comorbid conditions and control subjects.

IL18 −607C/A (rs1946518)				
Genotype frequency				
	Control individuals (%) (n = 49)	**CVD + Diabetes counts (%) (n = 36)**	**CVD + Hypertension counts (%) (n = 52)**	**CVD + Dyslipidemia counts (%) (n = 54)**
CC	39 (79.6)	31 (86.1)	44 (84.6)	49 (90.7)
CA	1 (2.0 %)	0 (0.0)	0 (0.0)	0 (0.0)
AA	9 (18.4)	5 (13.9)	8 (15.4)	5 (9.3)
P-value		**< 0.05**	NS	NS
Allele frequencies				
C	79 (80.6)	62 (86.1)	88 (84.6)	98 (90.7)
A	19 (19.4)	10 (13.9)	16 (15.4)	10 (9.3)
P-value		NS	NS	NS

Data are expressed as absolute numbers and percentages in parentheses. GG: wild-type, CC: homozygote; GC: heterozygote. The chi-square test was used for comparative analysis, and p-value < 0.05 was considered a significant criterion. NS: non-significant.

Table 4
Distribution of genotype and allele frequencies of IL 18–656 T/G (rs1946519) polymorphism in CVD comorbid conditions and control subjects.

IL18 −656 T/G (rs1946519)				
Genotype frequency				
	Control individuals (%) (n = 41)	**CVD + Diabetes counts (%) (n = 33)**	**CVD + Hypertension counts (%) (n = 49)**	**CVD + Dyslipidemia counts (%) (n = 52)**
GG	14 (34.1)	18 (54.5)	23 (46.9)	25 (48.0)
TG	17 (41.5)	10 (30.3)	18 (36.8)	22 (42.4)
TT	10 (24.4)	5 (15.2)	8 (16.3)	5 (9.6)
P-value		**< 0.001**	**< 0.001**	**< 0.001**
Allele frequencies				
G	45 (54.9)	46 (69.7)	64 (65.3)	72 (69.2)
T	37 (45.1)	20 (30.3)	34 (34.7)	32 (30.8)
P-value		**< 0.001**	**< 0.001**	**< 0.001**

Data are expressed as absolute numbers and percentages in parentheses. GG: wild-type, CC: homozygote; GC: heterozygote. The chi-square test was used for comparative analysis, and p-value < 0.05 was considered a significant criterion.

24.4 % TT genotype with 54.9 % G allele and 45.1 % T allele in control subjects. The CVD with diabetic comorbidity showed 54.5 %, 30.3 %, and 15.2 % GG, GT, and TT genotype, respectively. Whereas the allele frequencies of CVD with diabetic comorbidity were 69.7 % G and 30.3 % T. Similarly, the association of CVD with hypertension showed genotypic pattern as 46.9 % GG, 36.8 % GT and 16.3 % TT with an allele frequency of 65.3 % and 34.7 % of G and T, respectively. Moreover, the CVD association with dyslipidemia also showed highly significant changes with genotypic and allele frequency patterns as 48 % GG, 42.4 % GT, 9.6 % TT genotype and 69.2 % and 30.8 % of G and T, respectively.

3.5. Influence of CVD comorbidity and biochemical markers with IL-18 promoter genotyping

The genotypic pattern of IL-18 hotspots viz. −137 G/C (rs187238), −607C/A (rs1946518), and −656 T/G (rs1946519) were compared with comorbid conditions (diabetes, hypertension,

and dyslipidemia) and biochemical markers (Table 5). The GG, GC, and CC genotype of IL-18 at −137 G/C showed 72.1 %, 20.9 %, 7 % for diabetes, 61.6 %. 26.7 %, 11.7 % for hypertension, and 68.3 %, 20.6 %, 11.1 % for dyslipidemia respectively. Likewise, the CC, CA, and AA genotype of IL-18 at −607C/A showed 88.4 %, 0 %, 11.6 % for diabetes, 85 %. 0 %, 15 % for hypertension, and 91.8 %, 0 %, 8.2 % for dyslipidemia, respectively. On the other hand, the GG, GT, and TT genotype of IL-18 at −656 T/G showed 50 %, 35 %, 15 % for diabetes, 47.3 %, 38.2 %, 14.5 % for hypertension, and 46.6 %, 43.1 %, 10.3 % for dyslipidemia, respectively. The study also analyzed the biochemical parameters associated with CVD comorbidity viz. fasting blood sugar, HBA1c, total cholesterol, triglycerides, HDL-C and LDL-C are tabulated in Table 5. The genotypes pattern at all three-polymorphism hotspot did not show any statistical association with CVD comorbidities (diabetes, hypertension & dyslipidemia) and biochemical parameters except at −607C/A with dyslipidemia.

3.6. Mutual influence of different genotypic groups and their association with CVD comorbidity

Additionally, we analyzed the various genotypic subgroups associated with CVD comorbid diseases like diabetes, hypertension, and dyslipidemia (Table 6). The comparison was made between eighteen possible combinations of genotypic expressions (rs1946518 CC, rs1946519 GG, rs187238 GG); (rs1946518 CC, rs1946519 GG, rs187238 GC); (rs1946518 CC, rs1946519 GG, rs187238 CC); (rs1946518 CC, rs1946519 GT, rs187238 GG); (rs1946518 CC, rs1946519 GT, rs187238 GC); (rs1946518 CC, rs1946519 GT, rs187238 CC); (rs1946518 CC, rs1946519 TT, rs187238 GG); (rs1946518 CC, rs1946519 TT, rs187238 GC); (rs1946518 CC, rs1946519 TT, rs187238 CC); (rs1946518 AA, rs1946519 GG, rs187238 GG); (rs1946518 AA, rs1946519 GG, rs187238 GC); (rs1946518 AA, rs1946519 GG, rs187238 CC); (rs1946518 AA, rs1946519 GT, rs187238 GG); (rs1946518 AA, rs1946519 GT, rs187238 GC); (rs1946518 AA, rs1946519 GT, rs187238 CC); (rs1946518 AA, rs1946519 TT, rs187238 GG); (rs1946518 AA, rs1946519 TT, rs187238 GC); (rs1946518 AA, rs1946519 TT, rs187238 CC). We observed statistically significant ($p < 0.05$) association of dyslipidemia with three genotype combination (rs1946518 AA, rs1946519 GG, rs187238 GG); (rs1946518 AA, rs1946519 GT, rs187238 GG), and (rs1946518 AA, rs1946519 TT, rs187238 GG). All other genotypic combinations were found to be non-significant compared with CVD comorbidities.

4. Discussion

Cardiovascular disease often coexists with multiple comorbid conditions that could potentially promote critical myocardial damage and even death. Earlier studies suggested that patients with heart failure are often burdened by comorbid conditions, irrespective of age and sex (Buddeke et al., 2019). This area should be prioritized for future investigations to successfully manage CVD. According to Garcias-Olmos et al. (2018), diabetes and hyperlipidemia are the two conditions that CVD most frequently co-occurs with. Some other studies also determined that CVD usually co-occurs with hypertension, hyperlipidemia, diabetes, and arthritis (Murray et al., 2018, Ormazabal et al., 2018). The relevance of comprehending the relationships between CVD and comorbid conditions is highlighted by the rise in heart failure caused by non-cardiovascular diagnoses (Murray et al. 2018). However, the exact mechanisms underlying coronary complications and their relationship with comorbid conditions are still obscure.

Altered immune response and infiltration of pro-inflammatory cytokines have been considered the basis of chronic inflammation leading to atherosclerosis (Jabir et al., 2015, Jabir and Tabrez 2016). IL-18 has been suggested as an important inflammatory cytokine, associated with insulin resistance, endothelial dysfunction, and atherogenesis (Nwadiugwu 2021). Several scientific studies have indicated that variabilities in the genotype distribution of IL-18 polymorphisms affect the prevalence and clinical outcomes of CVD in a different ethnic population (Hoseini et al., 2018, Mitrokhin et al., 2018, Lian et al., 2019). Thus, we investigated the association of three IL-18 polymorphism sites involved in CVD with its comorbid conditions viz. diabetes, hypertension, and dyslipidemia. To the best of our knowledge, this is the first report on the possible association of IL-18 hotspots with CVD comorbidities in the Saudi population.

Our earlier studies reported a non-significant association of IL-18 promoter polymorphism with Saudi CVD patients. We also

Table 5
Influence of CVD comorbidity and associated biochemical markers with IL-18 promoter genotypes.

	IL18 −137 G/C (rs187238)				**IL18 −607C/A (rs1946518)**				**IL18 −656 T/G (rs1946519)**			
	GG	**GC**	**CC**	**P-value**	**CC**	**CA**	**AA**	**P-value**	**GG**	**GT**	**TT**	P-value
Diabetes	31 (72.1)	9 (20.9)	3 (7.0)	NS	38 (88.4)	0 (0.00)	5 (11.6)	NS	20 (50.0)	14 (35.0)	6 (15.0)	NS
Hypertension	37 (61.6)	16 (26.7)	7 (11.7)	NS	51 (85)	0 (0.00)	9 (15.0)	NS	26 (47.3)	21 (38.2)	8 (14.5)	NS
Dyslipidemia	43 (68.3)	13 (20.6)	7 (11.1).	NS	56 (91.8)	0 (0.00)	5 98.2)	**< 0.01**	27 (46.6)	25 (43.1)	6 (10.3)	NS
Fasting Blood Sugar (mmol/L)	8.79 ± 4.12	7.44 ± 3.26	8.66 ± 3.74	NS	8.44 ± 3.97	0 (0.00)	11.25 ± 4.43	NS	9.25 ± 4.58	7.24 ± 3.06	11.25 ± 4.43	NS
HBA1c (%)	8.44 ± 2.06	7.32 ± 1.40	9.12 ± 2.32	NS	8.25 ± 1.94	0 (0.00)	7.99 ± 2.75	NS	8.81 ± 2.19	7.59 ± 1.60	7.99 ± 2.75	NS
Total cholesterol (mmol/L)	4.42 ± 1.49	3.93 ± 0.67	3.68 ± 0.69	NS	4.28 ± 1.38	0 (0.00)	3.97 ± 0.76	NS	4.66 ± 1.59	3.79 ± 0.92	3.97 ± 0.76	NS
Triglycerides (mmol/L)	1.77 ± 1.15	1.62 ± 0.65	1.35 ± 0.28	NS	1.83 ± 1.10	0 (0.00)	1.24 ± 0.27	NS	1.96 ± 1.29	1.59 ± 0.83	1.24 ± 0.27	NS
HDL-C (mmol/L)	1.06 ± 0.25	1.09 ± 0.26	1.10 (0.32)	NS	1.06 ± 0.25	0 (0.00)	1.13 ± 0.24	NS	1.07 ± 0.26	1.04 ± 0.22	1.13 ± 0.24	NS
LDL-C (mmol/L)	2.96 ± 1.21	2.62 ± 0.54	2.21 ± 0.39	NS	2.15 ± 1.12	0 (0.00)	2.52 ± 0.57	NS	3.17 ± 1.30	2.44 ± 0.75	2.52 ± 0.57	NS

Data are expressed as absolute numbers and percentages in parentheses. GG: wild-type, CC: homozygote; GC: heterozygote. The chi-square test was used for comparative analysis, and p-value < 0.05 was considered a significant criterion. NS: non-significant.

Table 6
Mutual influence of genotypic groups and their association with CVD comorbid conditions.

	Groups	Diabetes (P-value)	Hypertension (P-value)	Dyslipidemia (P-value)
1	IL18 rs1946518 CC IL18 rs1946519 GG IL18 rs187238 GG	NS	NS	NS
2	IL18 rs1946518 CC IL18 rs1946519 GG IL18 rs187238 GC	NS	NS	NS
3	IL18 rs1946518 CC IL18 rs1946519 GG IL18 rs187238 CC	NS	NS	NS
4	IL18 rs1946518 CC IL18 rs1946519 GT IL18 rs187238 GG	NS	NS	NS
5	IL18 rs1946518 CC IL18 rs1946519 GT IL18 rs187238 GC	NS	NS	NS
6	IL18 rs1946518 CC IL18 rs1946519 GT IL18 rs187238 CC	NS	NS	NS
7	IL18 rs1946518 CC IL18 rs1946519 TT IL18 rs187238 GG	NS	NS	NS
8	IL18 rs1946518 CC IL18 rs1946519 TT IL18 rs187238 GC	NS	NS	NS
9	IL18 rs1946518 CC IL18 rs1946519 TT IL18 rs187238 CC	NS	NS	NS
10	IL18 rs1946518 AA IL18 rs1946519 GG IL18 rs187238 GG	NS	NS	**<0.05**
11	IL18 rs1946518 AA IL18 rs1946519 GG IL18 rs187238 GC	NS	NS	NS
12	IL18 rs1946518 AA IL18 rs1946519 GG IL18 rs187238 CC	NS	NS	NS
13	IL18 rs1946518 AA IL18 rs1946519 GT IL18 rs187238 GG	NS	NS	**<0.05**
14	IL18 rs1946518 AA IL18 rs1946519 GT IL18 rs187238 GC	NS	NS	NS
15	IL18 rs1946518 AA IL18 rs1946519 GT IL18 rs187238 CC	NS	NS	NS
16	IL18 rs1946518 AA IL18 rs1946519 TT IL18 rs187238 GG	NS	NS	<0.05
17	IL18 rs1946518 AA IL18 rs1946519 TT IL18 rs187238 GC	NS	NS	NS
18	IL18 rs1946518 AA IL18 rs1946519 TT IL18 rs187238 CC	NS	NS	NS

Data are expressed as absolute numbers and percentages in parentheses. GG: wild-type, CC: homozygote; GC: heterozygote. The chi-square test was used for comparative analysis, and p-value < 0.05 was considered a significant criterion. NS: non-significant.

observed a non-significant association of IL-18 gene promoter polymorphism with coronary artery lesions viz., single vessel disease and multi-vessel disease (Tabrez et al., 2021). In the present study, we extended the previous findings to investigate the associations of IL-18 promoter polymorphism hotspot at −137 G/C (rs187238), −607C/A (rs1946518), and −656 T/G (rs1946519) with CVD patients and one of the comorbid conditions. We also compared the genotype occurrence and single allele frequencies of the polymorphism hotspots in case-control groups. We observed a statistically significant association of GG, GC, and CC genotype at IL-18 gene locus rs187238 with the CVD-diabetes and CAD-hypertension group (Table 2). On the other hand, the CVD-dyslipidemia showed a positive association with allele distribution at IL-18 gene locus rs187238. These results indicate that the specific type of genotype at IL-18 gene locus rs187238 affects the susceptibility of CVD with comorbid conditions.

Several studies have investigated the possible association of IL-18 polymorphism at rs187238 with CVD, and conflicting findings have also been reported (Bazgir et al., 2018, Lian et al., 2019, Cavalcante et al., 2020). Previous scientific studies, including our lab, found no association between rs187238 hotspot polymorphism and CVD (Karič and Petrovič 2011, Jabir et al. 2017a, 2017b). However, Liu et al. (2009) observed a significant difference in the genotype distribution at rs187238 between CVD patients and the control groups (Liu et al., 2009). In another study, Ansari et al. (2017) reported a greater prevalence of rs187238 gene pro-

moter polymorphism in premature-CVD individuals compared to control subjects (Ansari et al., 2017). The scientific reports of an association between rs187238 gene promoter polymorphism in CVD patients with diabetes were also not consistent among different ethnic populations (Jabir et al., 2017a, 2017b, Cavalcante et al., 2020). However, numerous clinical investigations have demonstrated a positive correlation between IL-18 and its plasma levels and the pathogenesis and advancement of T2DM, hypertension, and CVD (ÖZzbïçer and Uluçam 2017, Zhuang et al., 2019).

Several studies have reported that IL-18 polymorphisms are associated with modulated expression level of concerned proteins in plasma. The polymorphisms in IL-18 hotspots (rs1946519, rs187238 and rs1946518) are associated with their transcription activity and plasma level (Lee and Bae 2016, Kadi et al., 2021). Hence, the current study tried to find possible associations of specific rs187238 genotypes, viz. GG, GC, CC in CVD-diabetic patients with control subjects and observed positive correlation (Table 2). A similar positive association of specific rs187238 genotypes with CVD with T2DM in the Brazilian population was reported earlier (Cavalcante et al., 2020). Additionally, Dong et al. (2017) revealed that Asians, but not Caucasians, are significantly more at risk for CVD due to the GG genotype of the IL-18–137 G/C polymorphism. The scientific community still needs to understand why genetic polymorphism studies provide conflicting results. However, the discrepancy can be explained by a number of factors, including variations in phenotype definitions between independent samples, variable selection criteria for the study population, variations in study design, heterogeneity in sample size, the existence of gene-gene and gene-environmental interactions, and different ethnic populations (Hoseini et al., 2018, Tabrez et al., 2022).

We also found a statistically significant association of GG, GT, and TT genotype and G and T allele distribution at rs1946519 (-656 T/G) IL-18 gene locus with CVD-diabetes, CVD-hypertension, and CVD-dyslipidemia group compared with control subjects (Table 4). IL-18 gene locus rs1946519 has also been reported to be associated with CVD in east Asian population and increased IL-18 plasma levels (Lee and Bae 2016, Zheng et al., 2021). To promote chronic inflammation these genotypes could increase the expression of plasma IL-18 leading to the development of CVD and associated complications. On the other hand, the genotype and associated allele at IL-18 gene locus rs1946518 did not show any association with CVD and comorbid conditions (Table 3). The current study also analyzed the possible associations of genotypes at IL-18 gene loci rs187238, rs1946518, and rs1946519 with different biochemical parameters associated with CVD comorbidity. The results showed no association between the specific genotype in CVD patients and studied biochemical parameters (Table 5). These findings may be explained by the fact that the pathogenesis of CVD is a diverse, complex process that is influenced by environmental variables in addition to hereditary ones, as in our case. According to Hoseini et al. (2018), the interaction of hereditary and environmental variables significantly accelerates CVD.

Due to the diversity of CVD pathogenesis, such as multiple genetic factors, gene-environment interactions, and gene-gene interactions, we also used a comparative analysis of the possible mutual influence of specific genotypes of rs187238, rs1946518 and rs1946519 hotspots with CVD comorbid conditions. We observed a statistically significant ($p < 0.05$) association of dyslipidemia with three genotype combination (rs1946518 AA, rs1946519 GG, rs187238 GG); (rs1946518 AA, rs1946519 GT, rs187238 GG) and (rs1946518 AA, rs1946519 TT, rs187238 GG) (Table 6). On the other hand, other possible genotype combinations did not show a statistically significant association compared with CVD comorbidities. As far as our knowledge goes from the available scientific literature, no study focused on the influence of IL-18 polymorphism hotspots with CVD comorbidities. However, the underlying mechanism needs to be explored. Because a significant portion of CVD management may be obtained by prevention, prompt recognition, and appropriate treatment, understanding the relationship between comorbidities and CVD may be useful for the care of specific patients.

The genetic polymorphisms in the promoter or regulatory regions of cytokines can control immunological function by regulating the expression of cytokines. Depending on the genetic composition, the variable expression of cytokine might be observed in an individual leading to a variety of inflammatory reactions (Zafar et al., 2019). Uncovering the genetic variations linked to CVD may advance our knowledge of the disease's pathophysiology and reduce the disease burden at both individual and population levels. Furthermore, genetic association studies may provide information, such as identifying high-risk individuals, identifying new pharmacologic targets, and pharmacogenomics (Hoseini et al., 2018).

The limitations of the present study are relatively small sample size, which may give false-positive or false-negative results in genetic association studies. Moreover, drugs such as statins could also downregulate the inflammatory process. Despite the above-mentioned limitation, our findings provide a clue for the possible associations between the specific genotype of IL18 polymorphism hotspots and CVD with comorbid conditions in Saudi population.

5. Conclusion

Cardiovascular disease is one of the most clinically relevant pathologies that remains the largest single contributor to global mortality. It is often associated with more than one comorbid condition, which obscures its clinical outcome. The results of current study showed that the GG, GC, and CC genotype at IL-18 gene locus, rs187238 has a statistically significant association with CVD-diabetes and CVD-hypertension group. On the other hand, rs1946519 loci showed a positive association with CVD-dyslipidemia. We also observed a statistically significant association of dyslipidemia with three genotypic combinations viz. (rs1946518 AA, rs1946519 GG, rs187238 GG); (rs1946518 AA, rs1946519 GT, rs187238 GG), and (rs1946518 AA, rs1946519 TT, rs187238 GG). Based on our study, we conclude that IL-18 loci, rs1946519 has a significant association with each studied comorbid conditions and can be considered a prognostic marker of CVD and comorbidities. However, the study on a bigger cohort is recommended so that a statistically significant association with different comorbid conditions might be established. Our findings are expected to be used to initiate a large pharmaco-genomic analysis that could differentiate prone CVD patients with comorbidities and also provide new genotypic perspectives on the influence of comorbidities on CVD progression.

6. Compliance with ethical standards

None declared.

Ethical approval

The current study was conducted in compliance with the Declaration of Helsinki and the study protocol was approved by the institutional ethical committee (Reference No. 84–16).

Author contributions

S.T, T.A.Z and N.R.J performed the experiments. M.S. was involved in the analysis of the sequenced data. All authors reviewed the final draft of manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The Deanship of Scientific Research (DSR) at King Abdulaziz University (KAU), Jeddah, Saudi Arabia has funded this project under grant no. (**G: 286-141-1443**).

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