Journal of Wasit for Science and Medicine 2026: 19, (2), 37-46

Predicting Role of VKORC1 Gene and Cardiac Enzymes in Incidence of Cardiovascular Diseases

Zainab Dhiyaa Jabori

Department of Biology, College of Science, Wasit University, Wasit, Iraq.

Abstract

VKORC1 gene encodes the VKORC1 enzyme that resides in endoplasmic reticulum membrane, and responsible for activating and recycling of vitamin K. The current study investigates the frequency of VKORC1 gene polymorphism in patients with cardiovascular diseases, and relationship with levels of serum cardiac enzymes in Wasit province (Iraq). Genetic analysis shows the significant high frequency of VKORC1-1639G >A was seen among the GA genotype (48.33%) when compared to GG (38.33%) and AA (13.33%) genotypes. While significant reduction was reported in GA genotype (8.33%) in comparison with other GG (63.33%) and AA (20%) genotypes in healthy cases. Subsequently, the frequency of G allele in patient and healthy individuals was significantly higher than the frequency of A allele. Regarding cardiac enzymes. This study demonstrates the effect of VKORC1-1639G>A polymorphism on incidence of cardiovascular diseases as shown in increasing the frequency of GA genotype in study patients compared to healthy individuals.

Keywords: c.-1639G/A Polymorphism, SNP Genotyping, Creatine kinase, Heart biomarkers, and Natriuretic peptide.

1. Introduction

Epoxide reductases play a crucial role in the vitamin K cycle, specifically in regenerating reduced vitamin K, which is essential for the carboxylation of various proteins involved in coagulation and bone metabolism [1, 2]. This generation is catalysed by VKORC1, an endoplasmic reticulum enzyme that facilitates the conversion of vitamin K epoxide back to the hydroquinone form in addition to its critical role critical for post-translational modification.

Specifically, the γ-carboxylation of glutamate residues, in key proteins like clotting factors [3, 4]. Inhibition of VKORC1 by warfarin, a widely prescribed

oral anticoagulant, underscores its pharmacological significance and the intricate nature of its catalytic mechanism [5, 6]. The VKORC1 protein, encoded by the VKORC1 gene localized to chromosome 16p11.2, is an 18-kDa integral membrane protein highly expressed in hepatocytes [7].

Genetic polymorphisms within the VKORC1 gene significantly influence individual sensitivity to warfarin, thereby impacting effective dosing strategies [8]. The enzyme's function within the vitamin K cycle involves the recycling of vitamin K 2,3-epoxide to vitamin K hydroquinone, which is a necessary cofactor for γ-glutamyl carboxylase [9]. This catalytic function is facilitated by a C132-X-X-C135 motif within VKORC1, through a mechanism of thiol-disulfide exchange reaction, where the CXXC motif interacts with protein disulfide isomerase to allow electron transfer [10]. Beyond the well-established role in blood coagulation, VKORC1, along with the isozyme VKORC1-like (VKORCAL1).

Also, participate in broader redox homeostasis within the cell suggesting that a consistent topology across mammalian VKOR isoforms and bacterial homologs, characterized by a large loop between transmembrane domains one and two facing the endoplasmic reticulum lumen, and N- and C-terminal oriented towards the cytosol [10, 11]. The precise relationships between specific VKORC1 gene polymorphisms and intrinsic VKORC1 activity in the absence of warfarin, and how these variations may independently contribute to cardiovascular disease pathogenesis remains an area requiring further elucidation. Subsequently, the discovery of the gene encoding VKORC1 provided a molecular basis for understanding pharmacogenetic-guided dosing strategies to optimize therapeutic outcomes and minimize adverse events. In Iraq, a few recent numbers of studies have been conducted to estimation the effect of VKORC1 genetic polymorphism on warfarin dose requirement in a sample of a population [11].

Moreover, the investigation of relationship between the rs9923231 SNP in VKORC1 gene with incidence of COVID-19 [11, 12]. Hence, the study focusses on the investigation of frequency of VKORC1 polymorphism in patients with cardiovascular diseases, and the relationship with levels of serum cardiac enzymes in Wasit province (Iraq).

2. Materials and Methods

2.1. Samples

This study included 45 patients diagnosed with various cardiovascular diseases in addition to 30 healthy persons at

Wasit province during December 2025. Approximately 5ml of venous blood was sampled from individual under aseptic conditions using disposable syringe and divided equally into free and anticoagulant-EDTA tubes. While the anticoagulant whole blood was frozen directly to be examined molecularly later, the free-anticoagulant blood was centrifuged to obtain the sera that kept into labelled Eppendorf tubes for serology.

2.2. Serological Examination

Kits of quantitative enzyme-linked immunosorbent assay (SunLong Biotech, China) were served for serological measurement of four cardiac enzymes including cTn-I (Cat.No.SL0411Hu), cTn-T(Cat.No.SL2238Hu), CK(Cat.No.SL0537 Hu), and NT-ProCNP (Cat.No.3325Hu). Following the manufacturer instructions of each marker's kit, serum samples and kit contents were prepared at room temperature, processed, read at an optical density of 450 nm, and calculated using the standard curve in the Microsoft Office Excel software.

2.3. Molecular Examination

After placing samples in water bath, DNA was extracted following the gSYNCTM DNA extraction kit (Genaid, Taiwan). Checked for purity and concentration and used to preparation of Master Mix tubes through the forward and reverse primers for the c.-1639G>A [5′-GCC AGC AGG AGA GGG AAA TA-3′ and 5′-AGT TTG GAC TAC AGG TGC CT-3′] using the PCR- RFLP at a final volume 25µl. Then, thermal cycler system was utilized for amplification of DNAs as following. One cycle for initial denaturation of 5 minutes at 95 °C, 35 cycles for 3 seconds at 95 °C, annealing for 30 seconds at 59 °C and extension of 30 seconds at 72 °C. Another, one cycle for final extension for 7 minutes at 72 °C. After digestion of PCR product by the MSP1 restriction enzyme (1µl, 37 °C / 30 minutes. Agarose-gel (1.5%) electrophoresis was completed at 100 volt and 80 Am for 90 minutes to detect SNP genotype at a product size of 289 bp [13, 14].

2.4. Statistical Analysis

Chi-square (x2), one-way ANOVA, and 95% confidence interval (95% CI) were calculated through the GraphPad Prism software. While odds ratio (OR), relative risk (RR) and number needed to treat (NNT) were measured by the MedCalc statistical software. Also, significant differences in frequency of genotypes and alleles in cardiovascular patients and healthy in addition to the serum biomarkers

of study groups were estimated at p<0.05 [15].

3. Results

3.1. VKORC1 Gene Polymorphism

The significant high frequency of VKORC1-1639G>A (p<0.0258) was seen among the patients with GA genotype (48.33%) when compared to other genotypes, GG (38.33%) and AA (13.33%) as listed in table 1.

Table 1: Genotype frequency of VKO-RC1-1639G>A among the study patients.

Genotype	Patient (No. 60)		p-value	95% CI
	No.	%		
GG	23	38.33	0.0258	11.45 to 78.11
GA	29	48.33*		
AA	8	13.33		

While for healthy, significant reduction (p < 0.00891) was reported in GA genotype (8.33%) in comparison with other GG (63.33%) and AA (20%) genotypes as listed in table 2.

Table 2: Genotype frequency of VKORC1-1639G>A among the study healthy control.

Genotype	Healthy (No. 30)		p-value	95% CI
	No.	%		
GG	19	63.33**	0.00891	41.43 to 102.5
GA	5	8.33		
AA	6	20		

However, results of study groups shown that the GA genotype was significantly higher (p < 0.05) in patients (38.33%) than healthy (8.33%). Whereas the polymorphism of GG and AA genotypes was lowered significantly (p < 0.05) in patients (38.33% and 13.33%, respectively more than healthy individuals 63.33% and 20% respectively as listed in table 3.

Also, the risk (OR, RR) of GA genotype among the cardiovascular patients (4.6774, 1.2919) was significantly higher (p<0.05) than GG (0.3599, 0.8129) and AA (0.6154, 0.8951) genotypes as listed in table 4.

Table 3: Genotype frequency of VKORC1-1639G>A among the study healthy control.

Genotype	Total	Patient	Healthy	p-value	95% CI
GG	42	23 (38.33%)	19 (63 .33%) *	0.0272	0.1453 to 0.8912
GA	34	29 (48.33%) **	0.0053	0.0053	1.580 to 13.85
AA	14	8 (13.33%)	0.0414	0.0414	0.1922 to 1.9705

Table 4: Risk of genotype frequency of VKORC1-1639G>A among the study population.

Genotype	Total	Patient	Healthy	OR	RR	NNT	95% CI
GG	42	23	19	0.3599	0.8129	12.278 Benefit	13.119 (Harm) to ∞ to 4.182 (Benefit)
GA	34	29	5	4.6774 ****	1.2919 ****	9.616 (Harm)	3.817 (Harm) to ∞ to 18.527 (Benefit)
AA	14	8	6	0.6154	0.8951	23.467 Benefit	5.590 (Harm) to ∞ to 3.786 (Benefit)
P-value				0.0001	0.0008	-	-
95%CI				4.133 to 7.902	0.3637 to 1.636	-	-

Subsequently, the frequency of G allele in study patients (62.5%) as well as in healthy control individuals (71.67%) was

significantly higher ($p<0.0354$, $p<0.0249$) than the frequency of A allele 37.5% and 28.33%, respectively as shown in tables 5 and 6. Comparative statistical analysis of allele frequency among study groups revealed that the G allele was reduced significantly ($p<0.0434$) in patients (62.5%) when compared to healthy control individuals (71.67%). Whereas significant increase in A allele ($p<0.0421$) was observed in patients (37.5%) when compared to healthy control individuals (28.33%) in table 7. However, the risk (OR, RR) of A allele (1.5176, 1.0822) was significantly ($p<0.0001$) higher than those of G allele (0.6589, 0.9240) as listed in table 8.

Table 5: Allele frequency of VKORC1-1639G>A among the study patients.

Allele	Patient		p-value	95% CI
	No.	%		
G	75	62.5 *	0.0354	108.8 to 208.8
A	45	37.5		

Table 6: Allele frequency of VKORC1-1639G>A among the study healthy control.

Allele	Healthy		p-value	95% CI
	No.	%		
G	43	71.67 *	0.0249	225.3 to 325.3
A	17	28.33		

Table 7: Allele frequency of VKORC1-1639G>A among the study healthy control.

Allele	Total	Patient	Healthy	p-value	95% CI
G	118	75 (62.5%)	43 (71.67%) *	0.0434	8.827 to 125.3
A	62	45 (37.5%) *	17 (28.33%)	0.0421	25.34 to 91.17

Table 8: Risk of Allele frequency of VKORC1-1639G>A among the study population.

Allele	Total	Patient	Healthy	OR	RR	NNT	95% CI
G	118	75	43	0.6589	0.9240	31.289 Benefit	11.945 (Harm) to ∞ to 6.773 (Benefit)
A	62	45	17	1.5176 ****	1.0822 ****	31.289 (Harm)	6.773 (Harm) to ∞ to 11.945 (Benefit)
P-value				0.0001	0.0001	-	-
95%CI				4.367 to 6.544	0.002 to 2.008	-	-

3.1. Cardiac Enzymes

Concerning the serum cTn-I, significant elevation $p< 0.0314$, 95% CI, 1312 to 1810 was shown in the findings of study patients 371.76 ± 22.53 pg/ml when compared to values of healthy control 126.03 ± 7.12 pg/ml individuals as shown in figure 1.

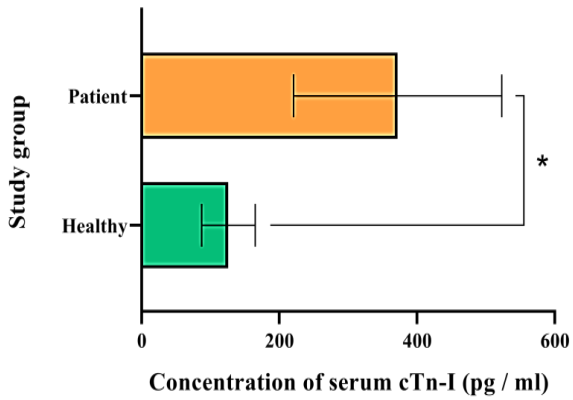

Figure 1: Results of serum cardiac enzyme

(cTn-I) among the study population.

Representing cTn-T, the study patients 198.82 ± 9.81pg/ml were significantly p<0.0021, 95% CI, 674.6 to 944.8 higher than recorded in healthy control individuals 71.37 ± 2.64 pg/ml, in figure 2.

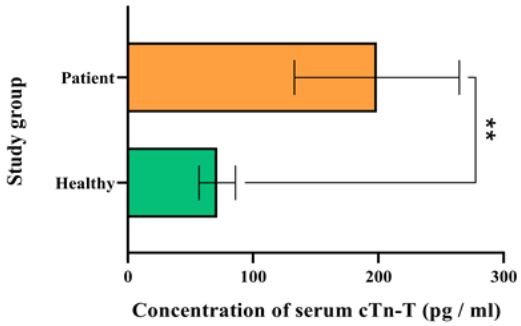

Figure 2: Results of serum cardiac enzyme (cTn-T) among the study population.

Concerning the serum CK, significant elevation p<0.0008, 95% CI, 4.210 to 56.50 was seen in values of study patients 8.22 ± 0.39 ng/ml in comparison with healthy control 3.32 ± 0.17ng/ml individuals in figure 3.

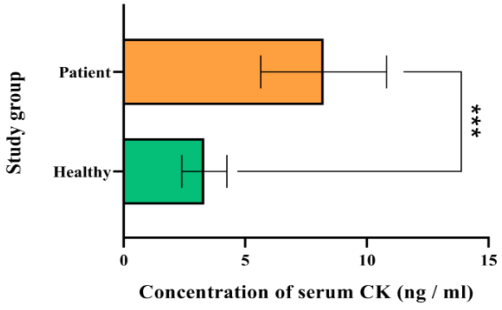

Figure 3: Results of serum cardiac enzyme (CK) among the study population.

Significant higher values of p<0.0001, 95% CI, 1.008 to 13.43 of NT-ProCNP were identified in study patients 2.6 ± 0.14pg/ml when compared to values of healthy control 0.75 ± 0.03 pg/ml individuals as shown in figure 4.

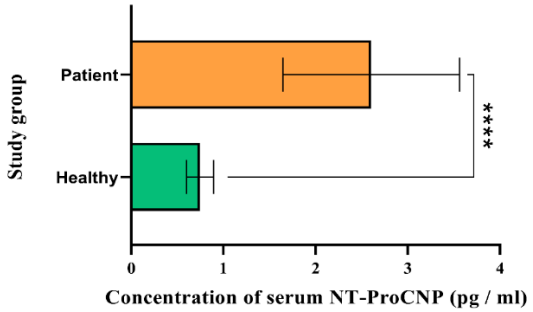

Figure 4: Results of serum cardiac enzyme (NT-ProCNP) among the study population.

4. Discussion

Cardiovascular diseases remain the leading cause of mortality with complex etiologist involving both environmental and genetic factors [16]. Genetic polymorphisms have emerged as significant contributors to individual susceptibility and pathogenesis of various cardiovascular conditions [17]. In current study, findings of genotyping were revealed a high significant elevation in VKORC1-1639G>A polymorphism among cardiovascular patients when compared to healthy individuals.

These results agreed with that reported by several studies conducted globally as the genetic variations in VKORC1 SNPs can extensively influence on

pharmacodynamics. Particularly impact inter-individual variability in dosage requirements, necessitating pharmacogenetic-guided dosing strategies to optimize therapeutic outcomes and minimize adverse events [16, 17]. In addition, the allelic variations influence gene transcription intensity and protein expression, thereby modulating the pathophysiological mechanisms underlying cardiovascular dysfunction [17, 18].

Moreover, genetic alter actions were strongly linked to the manifestation of ischemic stroke, arrhythmias, coronary artery disease, and myocardial infarction [18]. SNPs were demonstrated in critical pathways through regulation of blood pressure, vascular tone, renal sodium reabsorption, and oxidative stress, highlighting their integral role in cardiovascular pathology [19]. The accurate and timely diagnosis of acute cardiac pathologies is paramount for effective patient management and improved outcomes [18, 19]. In this study, serological measurement of cardiac enzymes involving cTn-I, cTn-T, CK and NT-ProCNP, revealed their relationships to cardiovascular diseases since the concentrations of markers were higher in study patients than those detected in healthy control individuals.

Ck has long been recognized as a pivotal biochemical marker in the assessment and diagnosis of acute myocardial infarction since 1960. However, the existence of specific isoenzyme such as CK-MB in heart improved the diagnostic precision and minimized false positives that arose from the presence of other CK isoenzymes in skeletal muscle [18-20]. Nonetheless, the utility of CK-MB as a sole diagnostic marker is tempered by its relative low specificity, as elevated levels can also manifest in various non-cardiac conditions affecting the liver, biliary tract, kidneys, and skeletal muscles [20, 21].

Also, cTns (I and T) have emerged as the gold biomarkers for identifying acute cardiac diseases particularly myocardial infarction in the 1990, owing to their superior diagnostic and prognostic capabilities compared to conventional and less specific markers [20, 21]. As proteins, cTns are integral to muscular contraction process, with specific isoforms being uniquely expressed in cardiac tissue making them invaluable for risk stratification in patients with suspected acute coronary syndrome [22]. Despite several clinical utilities, their individual limitations necessitate the exploration of novel indicators to enhance diagnostic precision and patient stratification [22, 23].

Among these, NT-ProCNP is developed as a promising candidate, warranting comprehensive investigation into its diagnostic capabilities for various

cardiovascular pathologies [22, 23]. Various studies mentioned that the discovery of NT-ProCNP participates significantly in advancing of heart failure diagnosis and assessing the prognostic risk particularly in conditions like acute and chronic coronary syndromes due to its physiological actions related to vascular tone regulation and cellular proliferation [23, 24].

5. Conclusion

This study demonstrates the effect of VKORC1-1639G>A polymorphism on incidence of cardiovascular diseases as shown in increasing the frequency of GA genotype in study patients compared to healthy individuals. However, the prevalence and specific associations of these genetic polymorphisms can vary significantly among different ethnic groups and populations necessitating further research to delineate population-specific risk profile. Subsequently, relationship between the patients and cardiac enzymes was related suggesting the importance of these biomarkers in estimation the risk of various cardiovascular diseases.

6. References

1. Arcidiacono G. P., Campello E., Simion C., Giannini S., and Simioni P., (2025). The underexplored crosstalk between hemostasis and bone metabolism: from mechanisms to clinical implications. Journal of thrombosis and haemostasis. 23, 11, 3424-3436.
2. Aldhufairi A., Alenezi D., Ebrahim E., Khalaf A., and Alkhabbaz A., (2025). A Narrative Review of the Genetic Architecture of Systemic Hypertension: From Candidate Genes to Biobank-Scale Genome-Wide Association Studies and Sequencing, With Translational Pathways to Precision Care. Cureus. 17, 12, e99825.
3. Al Hamad M., (2025). The Genetic Polymorphisms of CYP2C9 and VKORC1 in the Saudi Population and Their Impact on Anticoagulant Management. Medicina. 61, 10, 1872.
4. Banavandi M. J. S., and Satarzadeh N., (2020). Association between VKORC1 gene polymorphism and warfarin dose requirement and frequency of VKORC1 gene polymorphism in patients from Kerman province. The Pharmacogenomics Journal. 20, 4, 574-578.
5. Al-Fattli H. H. H., and Abd-Alraoof T. S., (2022). Investigation of Relationship Between (rs9923231) SNP in (VKORC1) Gene with Incidence of COVID-19. Journal of Pharmaceutical Negative Results. 13, 2, 23-31.
6. Al-Hassani M. K. A., Al-Gharban H. A., and Manher L. F., (2018). Application

of three diagnostic serologic techniques to detect of dromedary camel's brucellosis. Al-Qadisiyah journal for pure science. 23, 2, 61-74.

7. Binzamil J. M., (2025). A Comparative Study of Cardiac Biomarkers (Troponin I, CK-MB, and BNP) in Early Diagnosis of Acute Coronary Syndrome. Vascular and Endovascular Review. 8, 8s, 372-377.
8. Caspers M., Czogalla K. J., Liphardt K., Müller J., Westhofen P., Watzka M., and Oldenburg J., (2015). Two enzymes catalyze vitamin K 2, 3-epoxide reductase activity in mouse: VKORC1 is highly expressed in exocrine tissues while VKORC1L1 is highly expressed in brain. Thrombosis Research. 135, 5, 977-983.
9. Bermejo-Nogales A., Martín J. A. R., Coll J., and Navas J. M., (2022). VKORC1 single nucleotide polymorphisms in rodents in Spain. Chemosphere. 308, 136021.
10. Cao Z., Van Lith M., Mitchell L. J., Pringle M. A., Inaba K., and Bulleid N. J., (2016). The membrane topology of vitamin K epoxide reductase is conserved between human isoforms and the bacterial enzyme. Biochemical Journal. 473, 7, 851-858.
11. Cerrone M., Remme C. A., Tadros R., Bezzina C. R., and Delmar M., (2019). Beyond the one gene–one disease paradigm: complex genetics and pleiotropy in inheritable cardiac disorders. Circulation. 140, 7, 595-610.
12. Chatterjee K., Mazumder P. M., and Banerjee S., (2023). Vitamin K: a potential neuroprotective agent. Revista Brasileira de Farma-cognosia. 33, 4, 676-687.
13. Chen X., Liu Y., Furukawa N., Jin D. Y., Savage G. P., Stafford D. W., and Tie J. K., (2021). A novel vitamin K derived anticoagulant tolerant to genetic variations of vitamin K epoxide reductase. Journal of Thrombosis and Haemostasis. 19, 3, 689-700.
14. Chiasson M. A., Rollins N. J., Stephany J. J., Sitko K. A., Matreyek K. A., Verby M., and Fowler D. M., (2020). Multiplexed measurement of variant abundance and activity reveals VKOR topology, active site and human variant impact. Elife. 9, e58026.
15. Clerico A., Zaninotto M., Aimo A., Padoan A., Passino C., Fortunato A., and Plebani M. (2025). Advancements and challenges in high sensitivity cardiac troponin assays: Diagnostic, pathophysio-logical, and clinical perspectives: On behalf of the Italian Study Group on Cardiac Biomarkers. Clinical Chemistry and Laboratory Medicine (CCLM). 63, 7, 1260-1278.
16. Deepu V., Rai V., and Agrawal D. K., (2024). Interaction between genetic and

environmental factors in the pathogenesis of cardiovascular disease. In Envir-onmental Factors in the Pathogenesis of Car-diovascular Diseases. Springer Cham Nature Switzerland. 351-382.

17. Sitinjak B. D. P., Murdaya N., Rachman T. A., Zakiyah N., and Barliana M. I., (2023). The Potential of Single Nucleotide Polymorphisms (SNPs) as Biomarkers and Their Association with the Increased Risk of Coronary Heart Disease: A Systematic Review. Vascular health and risk management. 19, 289-301.
18. Fiatal S., and Adany R., (2018). Application of single-nucleotide polymorphism-related risk estimates in identification of increased genetic susceptibility to cardiovascular diseases: a literature review. Frontiers in public health. 5, 358.
19. GCRC (Global Cardiovascular Risk Consor-tium), (2023). Global effect of modifiable risk factors on cardiovascular disease and mortality. New England Journal of Medicine. 389, 14, 1273-1285.
20. Gharban H. A., (2024). First genotyping confirmation of Pichia kudriavzevii in sub-clinically mastitic cows in Iraq. Revista de Ciências Agroveterinárias. 23, 3, 417-424.
21. Ibrahim N., and Januzzi J. L., (2015). The potential role of natriuretic peptides and other biomarkers in heart failure diagnosis, prognosis and management. Expert review of cardiovas-cular therapy. 13, 9, 1017-1030.
22. Gruson D., Hammerer-Lercher A., Collinson P., Duff C., Baum H., Pulkki K., and Bayes-Genis A., (2024). The multidimen-sional value of natriuretic peptides in heart failure, integrating laboratory and clinical aspects. CritiCal reviews in CliniCal laboratory Sciences. 61, 6, 458-472.
23. Aldhaeefi M., Badreldin H. A., Alsuwayyid F., Alqahtani T., Alshaya O., Al Yami M. S., Bin Saleh K., Al Harbi S. A., and Alshaya A. I., (2023). Practical Guide for Anticoagulant and Antiplatelet Reversal in Clinical Practice. Pharmacy (Basel, Switzerland). 11, 1, 34.
24. Król M., and Kepinska M., (2020). Human nitric oxide synthase Its functions, polymorphisms, and inhibitors in the context of inflammation, diabetes and cardiovascular diseases. International journal of mole-cular sciences. 22, 1, 56.