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Association of KALRN gene polymorphism SNP rs9289231 and Kalirin serum levels with early-onset coronary artery disease: a case-control study in Iraq

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Abstract Coronary artery disease (CAD) is a major global contributor to mortality and morbidity. Genetic susceptibility plays a significant role in its etiology, particularly when CAD occurs prematurely. However, the relationship between premature CAD (PCAD) and specific genetic polymorphisms in the Iraqi population is still unclear. This aim of this study to assess the relationship between the KALRN gene single nucleotide polymorphism (SNP) (rs9289231T>G) and the development of PCAD in Iraqi patients. It also assesses the potential of serum Kalirin protein levels as a biomarker for PCAD. The study included 92 participants divided into two groups. Polymerase chain reaction (PCR) was performed to detect the KALRN SNP (rs9289231T>G), and the PCR products were analyzed via Sanger sequencing. Kalirin protein serum levels were measured using the ELISA technique. An association with statistical significance was found between the frequencies of genotypes and alleles of the KALRN SNP (rs9289231T>G) among the study groups, following a dominant genetic model. There was also a relationship between the genotypes of the KALRN SNP and CAD severity. Kalirin serum levels were significantly elevated in participants with CAD and were associated with the KALRN SNP genotypes. The KALRN SNP (rs9289231T>G) is associated with premature CAD in the Iraqi population, and follows a dominant genetic model. Elevated Kalirin serum levels, linked to the presence of the mutant allele, may serve as a biomarker for early diagnosis of the disease.

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Keywords: *coronary artery disease, genetic polymorphism, Kalirin, KALRN gene, premature coronary artery disease*

females at less than 55 years of age and in males at less than 45 years of age[8].

In Iraq, knowledge is poor regarding risk factors for CAD[9]. Increased prevalence of modifiable risk factors for CAD among young people includes low physical activity, smoking, and obesity[10]. The mentioned risk modifiers for the development of CAD may be different from the risk factors associated with angiographic severity[11]. This increases the demand for the use of additional biomarkers that correlate with coronary angiography findings as risk stratification to predict CAD severity independent of traditional risk factors[12].

Several genetic mutations linked with CAD development have been discovered by genome-wide association studies[13]. These genetic variations can serve as genetic biomarkers for CAD[14]. One of these genetic factors is the Kalirin (KALRN) gene[15]. Kalirin is a protein encoded by the KALRN gene that functions as a guanine nucleotide exchange factor for the GTPases Rac1 and RhoaA. Kalirin activity may contribute to atherogenesis by promoting the proliferation and migration of

1. INTRODUCTION

Coronary artery disease (CAD) is a disease from the group of cardiovascular diseases (CVD) and is a significant global health concern, contributing to high morbidity and mortality rates worldwide[1]. In Iraq, following data from the World Health Organization, CVD was a leading cause of mortality, accounting for over 18.5% of total mortality in 2017, placing Iraq nineteenth in the global rankings[2]. numerous risk factors contribute in the development of CAD, some of which can be modified, including increased blood pressure, increased blood lipid profile, consuming cigarettes, diabetes mellitus, increased body weight, lack of physical activity and stress[3]. In most of the cases, the cause of CAD is atherosclerosis[4]. Genetic susceptibility also plays a major role in increasing CAD risk and understanding the genetic basis of the disease is key[5]. Predisposition to CAD is equally divided between the presence of traditional risk factors and genetic factors[6]. This effect is even more pronounced when discussing early-onset coronary artery disease which also known as premature coronary artery disease (PCAD)[7], which is described as the onset of CAD in

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Body mass index (BMI) calculation was as follows: the weight of participant in kilograms divided by their square height in meters (kg/m2)[25]. Current smokers were defined as currently smoking one or more cigarettes per day. Past medical history was obtained from the patient's medical records regarding the presence and absence of hypertension, diabetes, dyslipidemia, and family history. These were confirmed by directly asking the participants.

Sample collection.

From each participant, samples of venous blood of four milliliters were drawn. Two milliliters were transferred into an EDTA (Ethylenediaminetetraacetic acid) tube and used for DNA extraction. The other two milliliters were drawn into a gel tube, left at room temperature for 30 minutes for clotting, and centrifuged for ten minutes at 4000 rpm to obtain serum. The resultant serum was stored in Eppendorf tubes for the measurement of Kalirin levels, using enzyme-linked immunosorbent assay (ELISA) technique. Both samples were stored frozen at -20 ℃ until enough samples were collected.

Serum Kalirin levels were measured using ELISA kits from Bioassay Technology Laboratories, China. The procedure employed the quantitative sandwich ELISA technique and was conducted according to the directions of the manufacturer[26,27]. The absorbance of the samples was recorded using a microplate reader (BioTek, USA) at 450nm wavelength[28], The concentration of the samples was calculated after plotting a standard curve and results for the concentration was described as ng/L.

DNA extraction and Genotyping

A ready commercial DNA extraction kit from Geneaid, Taiwan, was used for genomic DNA extraction, according to the instruction of the manufacturer[29]. The extracted DNA quality and quantity were measured using a nano-drop spectrophotometer (ScanDrop, Germany). The purity of the extracted DNA was determined by measuring the absorbance ratio at 260 and 280, and samples with extraction ratios less than 1.7 were re-extracted[30].

The primers for polymerase chain reaction (PCR) were designed using NCBI Primer-BLAST online software[31-33], supplied by Macrogen, Korea in lyophilized form, and prepared according to instructions, the primer sequence and length are shown in Table 1 , PCR was performed using a thermal cycler device (Hybaid, England), and the master mix solution was from Bioneer, Korea. The PCR mixture was prepared by adding 2 µL of extracted DNA, 1 µL of each primer, 10 µL of PCR master mix solution (Macrogen, Korea) and 6 µL of PCR-grade water, bringing the final volume to 20 µL.

Primer optimisation for conventional PCR was tested at various temperatures, and the optimal annealing temperature at 63 ℃ was determined after PCR amplification[34]. The products were assessed by agarose gel electrophoresis in 1% vascular smooth muscle cell (SMC) as well as endothelial dysfunction, among other mechanisms[16].

The KALRN gene has been studied in many atherosclerotic diseases, such as stroke[17], intracranial atherosclerotic stenosis[18], venous thromboembolism[19] and CAD. The single nucleotide polymorphism (SNP) rs9289231 is a candidate polymorphism associated with the disease[15,20].

The study aims to inspect the association between KALRN gene SNP rs9289231 and the development of PCAD in Iraqi patients and the assessment of serum Kalirin protein levels as a biomarker for PCAD

2. METHODS

Study design and participants.

The study is cross-sectional design and was carried out at Baghdad Heart Center in Baghdad Teaching Hospital, Baghdad Medical City in Baghdad, Iraq, between June 2022 and June 2023. Participants (n=92) were divided into two groups. The PCAD group (n=46) included patients diagnosed with PCAD, defined as 50% or more occlusion in one or more coronary arteries on coronary angiography, with ages <45 years old in males and <55 years in females[21]. The non-CAD group (n=46) included patients in the same age range who had no coronary artery occlusion, as confirmed by coronary angiography. The diagnosis process was supervised by a specialized cardiologist according to guideline published from the American Heart Association/American College of Cardiology.[22-24].

Inclusion criteria

Patients undergoing coronary angiography procedures, who were less than 45 years if males or less than 55 years old if females, were considered for enrolment in the study. Group allocation was based on angiographic results.

Exclusion criteria

Patients with Congenital heart disease, cardiomyopathy or valvular heart disease, were excluded from the study.

Ethical consideration

Ethical approval with the number (RECAUBCP1212022) was obtained on 12th of January 2022 from The Scientific and Ethical Committee in College of Pharmacy – University of Baghdad. Informed consent from all participants was obtained for this study and this work was compliant with the declaration of Helsinki and its subsequent amendments.

Data collection

The following participants' demographic and clinical data were collected at enrolment using a data sheet designed for this purpose: gender, age, height, weight, smoking status, past relevant medical history, CAD family history, results of angiographic procedures, and number of occluded arteries.

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The most prevalent genotype of the rs9289231 variant in both the CAD and non-CAD groups was the wildtype (T/T) genotype, which represented 78.3% of the non-CAD group and 56.5% of the CAD group.

Significant association found between the distribution of genotypes and alleles frequencies in the PCAD group (p-value < 0.05) as shown in Table 4.

Table 5 illustrates the three genetic models considered during the analyses to determine the relationship between the rs9289231 polymorphisms and study groups. KALRN gene (rs9289231) followed the dominant genetic model, in which participants with GG plus TG genotypes were more likely to develop PCAD compared to the TT genotype (OR and 95% confidence interval (95% CI); 2.768 (1.113-6.889), p-value > 0.05).

Table 6 illustrates the significant association between PCAD disease severity and genotype (p-value < 0.05).

Table 7 shows that serum Kalirin levels were significantly higher in the PCAD group compared to the non-CAD group (p-value < 0.05).

Table 8 shows that serum Kalirin levels were significantly associated with genotype in the non-CAD group and the CAD group (both p-values > 0.05).

4. DISCUSSION

The genetic influence on the development and severity of CAD has been the focus of several studies in scientific literature, highlighting the critical role that hereditary factors play in this disease[36,37]. However, this genetic effect is expected to be more pronounced than traditional risk factors in the case of PCAD, where individuals are affected at a younger age[7,38]. Understanding genetic predisposition is crucial; this could lead to targeted prevention strategies and personalized management approaches. Additionally, the identification of new serum biomarkers for risk stratification can help identify patients at risk, who may benefit from revascularization procedures [39,40].

This study tries to establish the association between KALRN gene SNP (rs9289231T>G) and PCAD and the plausibility of the association of its product, the Kalirin protein, with the development of PCAD in a sample of the Iraqi population.

In the current study, the CAD and non-CAD groups showed similar demographic characteristics, but the CAD group had significantly more participants who smoked (p=0.006) or who were diagnosed with diabetes (p=0.011) or dyslipidaemia (p=0.004). These findings are similar to those of many studies that compared a group of CAD patients with controls[41-43]. However, despite hypertension being a condition often observed in the elderly[44], no difference was observed among study groups in terms of the presence of hypertension, which is

concentration using ethidium bromide as a staining agent and viewed under ultraviolet light[35].

The PCR products were sent to Korea for sequencing using the Sanger method by DNA analyser (ABI3730XL) (Macrogen, Korea). The data received were analysed using Geneious Prime software (V 20201.1.1) and the genotype of rs9289231 was determined based on the peak present at position 330 on the DNA amplicon. A single “T” peak (green line) was identified as a homozygous T allele, the heterozygous T/G allele was identified when both “T” and “G” peaks were present (green and yellow lines), and the presence of a homozygous mutant (G/G) allele was identified by a single “G” peak (yellow line; see Figure 2.).

Statistical analysis

The SPSS software version 26 for Windows (SPSS, IL, USA) was used for the statistical analysis of the results. The categorical variables for the demographic and clinical data were presented as frequencies and percentages, any relation between these variables was assessed using Chi-square test. For continuous variables, normality was assessed using the Shapiro-Wilk test, these variables are described as means and standard deviations. The means are compared using the Students t-test.

For the genetic data, the Hardy-Weinberg equilibrium (HWE) online calculator for two alleles was used to check the distribution of observed and expected genotypes. The Chi-square test for 2 x 2 tables and the Fisher-Freeman-Halton exact test for n x k tables were performed to check the distribution of the genotypes and alleles among the study groups, then the likelihood of developing PCAD was assessed using logistic regression analysis for three genetic models, and described as the odds ratio (OR) and 95% confidence interval (95%CI).

The association of serum Kalirin levels according to the study groups and the genotypes, for two groups the Mann-Whitney U test and for three groups the Kruskal-Wallis test was used, and association with disease severity was tested with the Fisher-Freeman-Halton exact test. P-values of less than 0.05 were considered statistically significant.

3. RESULTS

Table 2 shows the characteristics of enrolled participants according to the study groups. Age, BMI, and sex were similar between the two groups, while some clinical characteristics were similar, such as the presence of hypertension and family history of CAD. Other factors such as smoking status, diabetes, and dyslipidemia prevalence were significantly different (p-value < 0.05) between the groups.

Genotype distribution was not statistically different between the observed and the expected values in either the CAD group (p-value=0.506) and the non-CAD group (p-value=0.406), according to Hardy-Weinberg equilibrium as shown in Table 3.

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endothelial cells, and monocytes lead to altered interactions in injured arteries, making serum Kalirin levels a potential early marker for pre-atherosclerotic intimal hyperplasia[48].

When comparing the PCAD and non-CAD groups, the serum Kalirin levels in the PCAD group were significantly (p=0.001) higher than in the non-CAD group. These findings are similar to a study conducted in Iran[48]. Although the results found were not statistically significant, the CAD group had higher Kalirin levels than the control group in the Iranian study[48]. This association was also observed when comparing levels of Kalirin between rs9289231 genotypes in the non-CAD (p=0.049) and PCAD (p=0.015) groups.

Considering that KALRN SNP (rs9289231) is an intron mutation, two hypotheses relate to the observed results in the current study. The first hypothesis is that the intron mutation, although not directly expressed or transcribed in the target RNA or protein, can still affect gene transcription, and may sometimes boost gene transcription[51]. The second hypothesis is that a mutation in miRNA might yet be identified[52]. The investigation of these hypotheses is outside the scope of this study, but these assumptions may justify the observed results.

In summary, this study reported a link between the KALRN gene variant (rs9289231) and PCAD and the severity of CAD. Furthermore, serum Kalirin levels were associated with the development of PCAD and PCAD severity, and Kalirin showed a promising diagnostic ability as a serum biomarker for PCAD. Further research is required to identify additional variations in the KALRN gene and other genes that may have affected the development and severity of PCAD.

This study also has some limitations including the relatively small sample size, the case-control study design which can show association but fails to confirm causality, the study was conducted in a single center only, it was not possible to screen patients for further genes that may also be associated with CAD or further biomarkers to fully understand the relationship of KALRN with PCAD. It is recommended that these study findings can be further validated in future research with taking into account the limitations encountered by the researchers, including a multi-center prospective study design with a larger sample size and more genes or biomarkers.

5. CONCLUSION

This study has shown that KALRN SNP (rs9289231) is associated with the presence and development of PCAD and the gene product, the Kalirin protein is also associated with PCAD development and severity, and its serum levels may provide benefit to be used as an additional biomarker for PCAD in association with other biomarker. Further research is encouraged to confirm the results of the current study as well as to discover further genetic mutations associated with PCAD.

explained by the high prevalence of hypertension in young Iraqis[45,46].

No significant deviations from the HWE in the genotype frequency (p=0.506) and allele frequency (p=0.408) were observed, suggesting that the sample tested in genetic equilibrium and the observed genotype frequency are consistent with those expected in a random combination of alleles.

The study of genotype and allele frequency showed a significant difference (p=0.025, 0.013, respectively) between the CAD and non-CAD groups, which suggests a relationship between the SNP in question (rs9289231) and the development of PCAD. Only two research papers have observed this association directly in the Iranian population, and both reached similar results regarding the association of the genotypes and alleles frequency with PCAD[47,48]. Moreover, when assessing the association of KALRN SNP (rs9289231T>G) with the study groups according to three genetic models, only the dominant model showed a significant association (p=0.028) with PCAD and increased the risk of developing PCAD by 2.769-fold in comparison with the non-CAD group. This suggests that this SNP has a dominant effect and even the presence of one allele in the gene is sufficient to increase PCAD risk. These findings accord with the classification of the SNP in question in NCBI ClinVar as pathologic susceptibility SNP for CAD[49].

KALRN SNP (rs9289231T>G) genotype was also significantly associated (p=0.001) with PCAD severity classified according to the number of vessels affected, which suggests that patients with the risk allele not only have an increased risk of developing CAD but also a more severe CAD. This relationship was previously explored in an Iranian study of the frequency of alleles but not genotype with CAD severity and, similar to the current study, concluded that the minor mutant G allele is associated with higher CAD severity[47].

Kalirin, a guanine nucleotide exchange factor, is implicated in the development of atherosclerosis through its role in SMC signalling and motility via Rac-1 and Rho A activation. These proteins regulate SMC proliferation, migration, and adhesion[50]. Genetic variations in KALRN, particularly the rs9289231 SNP, are associated with CAD[15], suggesting the involvement of Kalirin in the Rho GTPase signalling pathway, as evidenced by the 2007 CATHGEN study[15].

Further research indicates that Kalirin influences SMC migration and proliferation by affecting the Rac-1 pathway and interacting with receptor tyrosine kinases and NOS2. Loss-of-function studies show that reduced Kalirin decreases SMC migration by lowering Rac-1 activation. Kalirin also promotes monocyte/macrophage infiltration, contributing to neointimal hyperplasia[16]. Varying ammounts of Kalirin in SMCs,

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Tables

Table 1. *primer characteristics.*

Primer Sequence	Length	GC%	Amplicon size (base pairs)
5'-TGGGTTGTGATTATCAGTAGTTCCA-3' 5'-TTTAGTGGCATCAGGAGCGG-3'	25 20	36% 55%	506

Table 2. *Assessment of socio-demographic and disease characteristics.*

Parameters	Non-CAD (n=46)	CAD (n=46)	p-value
Age (y), mean ± SD	42.28±5.82	42.46±5.12	0.879
BMI (kg/m^2), mean ± SD	27.13±5.37	28.07±4.31	0.362
Sex			0.662
Female, n (%)	17(37.0%)	15(32.6%)	
Male, n (%)	29(63.0%)	31(67.4%)	
Smoking, n (%)	13(28.3%)	26(56.5%)	0.006*
Hypertension, n (%)	26(56.5%)	30(65.2%)	0.392
DM, n (%)	13(28.3%)	25(54.3%)	0.011*
Dyslipidaemia, n (%)	17(36.9%)	31(67.9%)	0.004*
Family history of CAD, n (%)	22(47.8%)	27(58.7%)	0.296

Values presented as number (percentages), mean ± SD

Table 3. *Genotype distribution and adherence to Hardy – Weinberg equilibrium in KALRN gene.*

Genotype	Frequency (%)		P value
	Observed	**Expected**	
CAD group			
GG	4(8.7%)	3 (6.5%)	0.506
TG	16(34.8%)	18 (39.1%)	
TT (wild type)	26(56.5%)	25 (54.3%)	
Non-CAD group			
GG	0(0.0%)	1 (2.5%)	0.408
TG	10(21.7%)	9 (19.6%)	
TT (wild type)	36(78.3%)	36 (78.3%)	

Table 4. *Distribution of KALRN gene (rs9289231) SNP genotypes and allele frequency according to study groups.*

SNP rs9289231	Frequency (%)		p-value
Genotype	**Non-CAD (n=46)**	**CAD (n=46)**	
GG	0(0.0%)	4(8.7%)	0.025
TG	10(21.7%)	16(34.8%)	
TT (wild type)	36(78.3%)	26(56.5%)	
Allele			
T	82 (89.1%)	68 (73.9%)	0.013
G	10 (10.9%)	24 (26.1%)	

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Table 5. *Assessment of the association between KALRN gene (rs9289231) SNP polymorphism and study groups using genetic models.*

Genotype model	Frequency (%)		OR (95%CI)	p-value
	Non-CAD (n=46)	CAD (n=46)		
Co-dominant model				
GG	0(0.0%)	4(8.7%)	12.396 (0.64-240.2)[a]	0.086
TG	10(21.7%)	16(34.8%)	2.215 (0.868-5.657)	0.096
TT	36(78.3%)	26(56.5%)	Reference	
Dominant genetic model				
GG + TG	10(21.7%)	20(43.5%)	2.769 (1.113-6.889)	0.028*
TT	36(78.3%)	26(56.5%)	Reference	
Recessive genetic model				
TT + TG	46(100.0%)	42(91.3%)	5.465(0.624-48.663)[a]	0.204
GG	0(0.0%)	4(8.7%)	Reference	

*a: using Woolf-Haldane correction (correction was applied by adding 0.5 to each cell count if a zero was in at least one cell of the 2*2 table)*

Table 6. *Association between rs9289231 KALRN gene polymorphism and disease severity.*

	GG	TG	TT	p-value
Number	4	16	26	-
One vessel	0(0.0%)	6(37.5%)	13(50.0%)	<0.001
Two vessels	0(0.0%)	3(18.8%)	12(46.2%)	
≥3 vessels	4(100.0%)	7(43.8%)	1(3.8%)	

Table 7. *Assessment of Kalirin (ng/L) according to the study groups.*

Parameters	Non-CAD (n=46)	CAD (n=46)	p-value
Kalirin (ng/L), median (IQR)	201.6 (122.6 - 312.0)	312.8 (219.7 - 447.6)	<0.001

Values presented as median (interquartile range)

Table 8. Levels of Kalirin according to genetic polymorphism of KALRN gene (rs9289231) SNP.

Parameters	Non-CAD (n=46)	p-value	CAD (n=46)	p-value
GG	-	0.049*	569.1 (448.0-758.3)	0.015*
TG	307.4 (188.7- 379.7)		295.3 (209.1- 339.7)	
TT (wild type)	194.9 (116.4- 279.9)		355.8 (236.2- 488.5)	

Values presented as median (interquartile range)

Figures

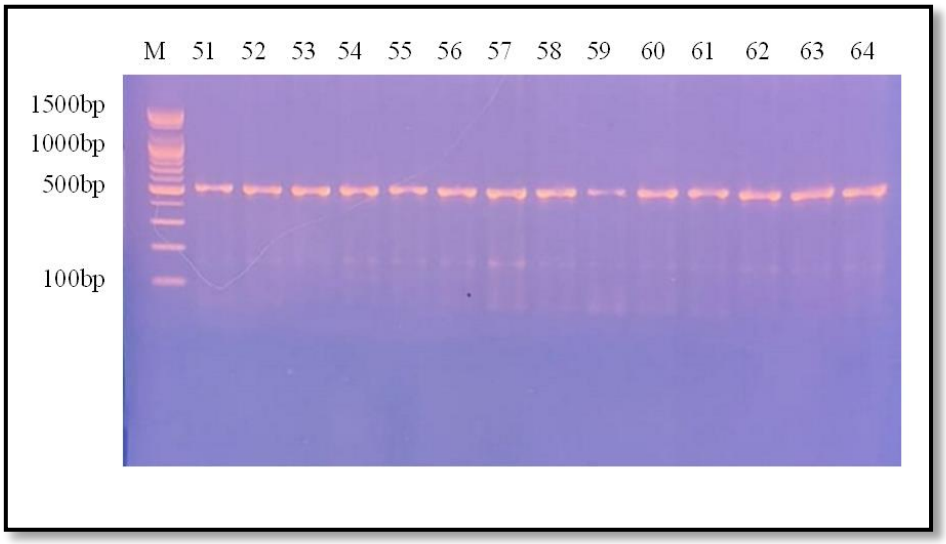

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Figure 1. *Gel Electrophoresis for the products of PCR amplification using Ethidim Bromide as indicator*

This figure illustrates Results of the PCR amplification of KARLN gene of the study samples that were fractionated on 1% agarose gel electrophoresis stained with Ethidium Bromide. M: 100bp ladder marker. Lanes 51-64: resemble 506 bp product

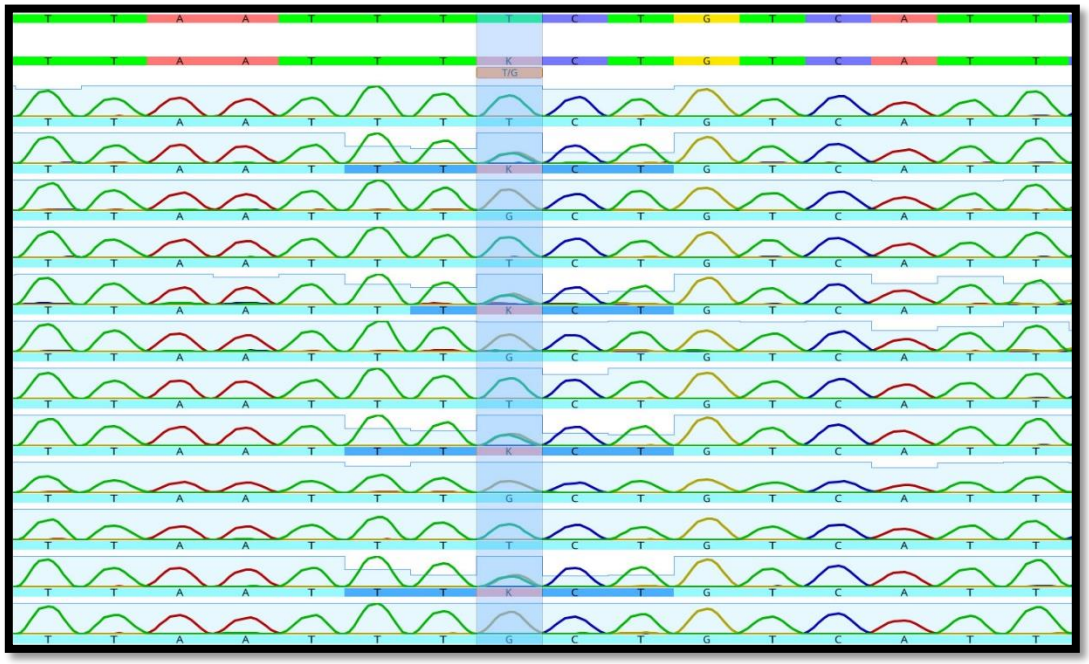

Figure 2. Sequencing and genotyping for KALRN gene SNP (rs9289231).

This figure illustrates the analysis of PCR products after sequencing using Geneious prime software (V 20201.1.1). the SNP location is highlighted with blue, the presence of a single green peak indicates TT homozygous genotype and is identified by the letter "T", the presence of a single yellow peak indicates the presence of GG homozygous genotype and is identified by the letter "G", the presence of overlapping green and yellow peaks indicates TG heterozygous genotype and is identified by the letter "K".

REFERENCES

[1] World Health, O. *Global status report on noncommunicable diseases 2014*. (World Health Organization, 2014).

[2] Al-Attar, M. M., Al-Awadi, S. J. A.-A. & Abdulfattah, S. Y. Gene Expression and Methylation Levels of PCSK9 Gene in Iraqi Patients with Coronary Artery Disease. *Baghdad Science Journal* (2023).

[3] Hajar, R. Risk Factors for Coronary Artery Disease: Historical Perspectives. *Heart Views* **18**, 109-114, doi:10.4103/HEARTVIEWS.HEARTVIEWS_106_17 (2017).

[4] Ahmed, H. S. & Saleh Mohammed, M. Atherogenic Indices in Type 2 Diabetic Iraqi Patients and Its Association with Cardiovascular Disease Risk. *Journal of the Faculty of Medicine Baghdad* **65**, 179-186, doi:10.32007/jfacmedbagdad.2075 (2023).

[5] Mohammed, W. J., Al-Musawi, B. M. S., Oberkanins, C. & Pühringer, H. Molecular assessment of some cardiovascular genetic risk factors among Iraqi patients with ischemic heart diseases. *International Journal of Health Sciences* **12**, 44 (2018).

[6] Roberts, R., Chavira, J. & Venner, E. Genetic risk and its role in primary prevention of CAD. (2022).

[7] Wang, H. *et al.* Pathogenesis of premature coronary artery disease: Focus on risk factors and genetic variants. *Genes Dis* **9**, 370-380, doi:10.1016/j.gendis.2020.11.003 (2022).

[8] Sharma, S. K. *et al.* Premature coronary artery disease, risk factors, clinical presentation, angiography and interventions: Hospital based registry. *Indian Heart J* **74**, 391-397, doi:10.1016/j.ihj.2022.08.003 (2022).

[9] Majeed, A. H. J. H. M. & Mohammed, T. R. Knowledge and Protective Health Behaviors Concerning Risk Factors for Coronary Heart Disease among Baghdad University Students. *Medico-Legal Update* **20**, 234-239, doi:10.37506/mlu.v20i2.1108 (2020).

[10] Qadir, M. & M. Weli, S. Prevalence of cardiovascular disease risk factors among secondary school pupils in Sulaimani city Kurdistan-Iraq. A cross-sectional study. *Journal of the Faculty of Medicine Baghdad* **65**, doi:10.32007/jfacmedbagdad.2050 (2023).

[11] Al-rubae, S. M. Evaluating coronary artery disease in type 2 diabetes mellitus and Other Risk Factors by angiographic study. *Journal of the Faculty of Medicine Baghdad* **53**, 15-19, doi:10.32007/jfacmedbagdad.531901 (2011).

[12] AbdulKarim, N. G., Taher, M. A. & Almarayati, A. N. The role of some inflammatory markers (IL-6 and CRP) in the pathogenesis of acute coronary syndrome in Iraqi CCU for Heart Diseases. *Iraqi J Pharm Sci* **20**, 43-94 (2011).

[13] Khera, A. V. & Kathiresan, S. Genetics of coronary artery disease: discovery, biology and clinical translation. *Nat Rev Genet* **18**, 331-344, doi:10.1038/nrg.2016.160 (2017).

[14] Suleiman, A. A., Muhsin, H., Abdulkareem, R. A. & Abed, F. A. Association study of two single nucleotide polymorphisms rs10757278 and rs1333049 with atherosclerosis, a case-control study from Iraq. *Mol Biol Res Commun* **8**, 99-102, doi:10.22099/mbrc.2019.33818.1406 (2019).

[15] Wang, L. *et al.* Peakwide mapping on chromosome 3q13 identifies the kalirin gene as a novel candidate gene for coronary artery disease. *The American Journal of Human Genetics* **80**, 650-663 (2007).

[16] Wu, J. H. *et al.* Kalirin promotes neointimal hyperplasia by activating Rac in smooth muscle cells. *Arterioscler Thromb Vasc Biol* **33**, 702-708, doi:10.1161/ATVBAHA.112.300234 (2013).

[17] Dang, M. *et al.* KALRN Rare and Common Variants and Susceptibility to Ischemic Stroke in Chinese Han Population. *Neuromolecular Med* **17**, 241-250, doi:10.1007/s12017-015-8352-z (2015).

[18] Dang, M. *et al.* Genetic variation of the kalirin gene is associated with ICAS in the Chinese population. *Journal of Molecular Neuroscience* **66**, 157-162 (2018).

[19] Mateos, M. K. *et al.* Genome-Wide Association Meta-Analysis of Single-Nucleotide Polymorphisms and Symptomatic Venous Thromboembolism during Therapy for Acute Lymphoblastic Leukemia and Lymphoma in Caucasian Children. *Cancers (Basel)* **12**, 1285, doi:10.3390/cancers12051285 (2020).

[20] Mofarrah, M. *et al.* Association of KALRN, ADIPOQ, and FTO gene polymorphism in type 2 diabetic patients with coronary artery disease: possible predisposing markers. *Coron Artery Dis* **27**, 490-496, doi:10.1097/MCA.0000000000000386 (2016).

[21] Mohammad, A. M., Jehangeer, H. I. & Shaikhow, S. K. Prevalence and risk factors of premature coronary artery disease in patients undergoing coronary angiography in Kurdistan, Iraq. *BMC Cardiovasc Disord* **15**, 155, doi:10.1186/s12872-015-0145-7 (2015).

[22] Levine, G. N. *et al.* 2016 ACC/AHA guideline focused update on duration of dual antiplatelet therapy in patients with coronary artery disease: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines: an update of the 2011 ACCF/AHA/SCAI guideline for percutaneous coronary intervention, 2011 ACCF/AHA guideline for coronary artery bypass graft surgery, 2012 ACC/AHA/ACP/AATS/PCNA/SCAI/STS guideline for the diagnosis and management of patients with stable ischemic heart disease, 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction, 2014 AHA/ACC guideline for the management of patients with non–ST-elevation acute coronary syndromes, and 2014 ACC/AHA guideline on perioperative cardiovascular evaluation and management of patients undergoing noncardiac surgery. *Circulation* **134**, e123-e155 (2016).

[23] Members, W. C. *et al.* 2021 AHA/ACC/ASE/CHEST/SAEM/SCCT/SCMR guideline for the evaluation and diagnosis of chest pain: a report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Journal of the American College of Cardiology* **78**, e187-e285 (2021).

[24] Members, W. C. *et al.* 2023 AHA/ACC/ACCP/ASPC/NLA/PCNA guideline for the management of patients with chronic coronary disease: a report of the American Heart Association/American College of Cardiology Joint Committee on Clinical Practice Guidelines. *Journal of the American College of Cardiology* **82**, 833-955 (2023).

[25] Baral, P., Shrestha, R., Shrestha, R. N., Banstola, D. & Prajapati, R. A study of height, weight and body mass index in Nepalese. *Journal of Gandaki Medical College-Nepal* **14**, 88-92 (2021).

[26] *Human Kalirin, KALRN ELISA Kit - BT LAB*, <https://www.bt-laboratory.com/index.php/Shop/Index/productShijiheDetail/p_id/812.html> (

[27] Kumar, V., Gill, K. D., Kumar, V. & Gill, K. D. (Springer, 2018).

[28] Jabbar, T. M. Polymorphism of the Aromatase Enzyme Gene at the rs700519 Site and Its Relationship with Some Biochemical Variables in Women with Polycystic Ovary Syndrome. *Journal Port Science Research* **6**, 16-21 (2023).

[29] *Tissue/Blood DNA Mini Kit (GS100) - Geneaid Biotech Ltd*, <https://www.geneaid.com/Genomic-DNA-Purification/GS> (

[30] Hasan, E. K., Kasim, A. A. & Matti, B. F. Impact of MDR-1 Gene Polymorphism (rs1128503) on Response to Imatinib or Nilotinib in Iraqi Patients with Chronic Myeloid Leukemia: An Observational Study. *Al-Rafidain Journal of Medical Sciences (ISSN 2789-3219)* **6**, 215-221 (2024).

[31] Yahya, A. A., Kadhim, D. J. & Abdalhadi, N. A. The role of angiotensin converting enzyme (insertion)/(deletion) and angiotensin II type 1 receptor (A1166C) gene polymorphisms in antiproteinuric effect of ACE inhibitors in type 2 diabetic Iraqi patients. *Journal of Applied Pharmaceutical Science* (2024).

[32] *National Library of Medicine, Primer-Blast*, <https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi> (

[33] Mohammad, W. J., Ibrahim, N. A. & Obeid, S. F. Decreased expression of IL-4 Gene and Exploring of mutable lymphotoxin alpha (TNF-β) gene in Patients with Systemic Lupus Erythematosus. *Journal Port Science Research* **7**, 6-14 (2024).

[34] Mohammad, W. J., Ibrahim, N. A. K., Obed, S. F. & Jebur, M. S. Association of TNFRII polymorphisms and IL-37 in rheumatoid arthritis Iraqi patients. *Journal port Science Research* **4**, 35-40 (2021).

[35] Abbas, Z. F., Hasan, B. J., Al-Dean, S. L. M. S. & Mozdarani, H. Distribution of FSHR-29 polymorphism among women with polycystic ovary syndrome and association with level of its receptor expression in Granulosa cells of infertile women. *Journal Port Science Research* **6**, 4-10 (2023).

[36] Ashiq, S., Ashiq, K., Shabana, S. U. S., Qayyum, M. & Sadia, H. Prevalence and role of different risk factors with emphasis on genetics in development of pathophysiology of coronary artery disease (CAD). *Pakistan Heart Journal* **52** (2019).

[37] Roberts, R., Campillo, A. & Schmitt, M. Prediction and management of CAD risk based on genetic stratification. *Trends Cardiovasc Med* **30**, 328-334, doi:10.1016/j.tcm.2019.08.006 (2020).

[38] Le, A. *et al.* What Causes Premature Coronary Artery Disease? *Current Atherosclerosis Reports* **26**, 189-203 (2024).

[39] Bargieł, W. *et al.* Recognized and potentially new biomarkers—their role in diagnosis and prognosis of cardiovascular disease. *Medicina* **57**, 701 (2021).

[40] Wong, Y. K. & Tse, H. F. Circulating Biomarkers for Cardiovascular Disease Risk Prediction in Patients With Cardiovascular Disease. *Front Cardiovasc Med* **8**, 713191, doi:10.3389/fcvm.2021.713191 (2021).

[41] Goodarzynejad, H., Boroumand, M., Behmanesh, M., Ziaee, S. & Jalali, A. Cholesteryl ester transfer protein gene polymorphism (I405V) and premature coronary artery disease in an Iranian population. *Bosn J Basic Med Sci* **16**, 114-120, doi:10.17305/bjbms.2016.942 (2016).

[42] Goodarzynejad, H. *et al.* Association between the hepatic lipase promoter region polymorphism (-514 C/T) and the presence and severity of premature coronary artery disease. *The Journal of Tehran University Heart Center* **12**, 119 (2017).

[43] Low-Kam, C. *et al.* Variants at the APOE/C1/C2/C4 locus modulate cholesterol efflux capacity independently of high-density lipoprotein cholesterol. *Journal of the American Heart Association* **7**, e009545 (2018).

[44] Abbas, Z. & Manhal, F. Comorbidities and risk factors for COVID-19 in a group of Iraqi patients confirmed by real-time PCR test. *Journal Port Science Research* **4**, 1-5 (2021).

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[45] Al-Mashhadani, Z., Aboddy, A. A., Alhamdy, R. A., Aljawari, A. Y. & Aljawari, H. Y. Evaluation of Incidence, Causes, and Management of Hypertension among a Sample of Iraqi Young People. *International Journal of Pharmaceutical Research (09752366)* **13** (2021).

[46] Saka, M., Shabu, S. & Shabila, N. Prevalence of hypertension and associated risk factors in older adults in Kurdistan, Iraq. *East Mediterr Health J* **26**, 268-275, doi:10.26719/emhj.19.029 (2020).

[47] Boroumand, M. *et al.* The Kalirin gene rs9289231 polymorphism as a novel predisposing marker for coronary artery disease. *Laboratory medicine* **45**, 302-308 (2014).

[48] Shafiei, A., Pilehvar-Soltanahmadi, Y., Ziaee, S., Mofarrah, M. & Zarghami, N. Association between Serum Kalirin Levels and the KALRN gene rs9289231 polymorphism in early-onset coronary artery disease. *The Journal of Tehran University Heart Center* **13**, 58 (2018).

[49] *National Center for Biotechnology Information. ClinVar; RCV000005791.3*, <https://www.ncbi.nlm.nih.gov/clinvar/RCV000005791.3/> (

[50] Fanaroff, A. C. *et al.* (Am Heart Assoc, 2010).

[51] Rose, A. B. Introns as gene regulators: a brick on the accelerator. *Frontiers in genetics* **9**, 672 (2019).

[52] Ratnadiwakara, M., Mohenska, M. & Änkö, M.-L. in *Seminars in cell & developmental biology*. 113-122 (Elsevier).