International Journal of Health Disciplines
IJHD
Open Access
BJSOL
Research Article

Association analysis of single nucleotide polymorphisms (SNPs) in core promoter region of m6A writer protein in cardiovascular disease

Ayesha Faryal 1*

1 Department of Biochemistry and Molecular Biology, COMSATS University, Islamabad, Pakistan

***Corresponding author:** ayesha_faryal11@outlook.com

Citation:

Faryal A. (2026).Association analysis of single nucleotide polymorphisms (SNPs) in core promoter region of M^{6}a write protein in cardiovascular disease. *International Journal of Health Disciplines,1(1), 63-84*

Received: 22-09-2025

Revised: 14-03-2026

Accepted: 11-04-2026

Published: 30-06-2026

Abstract:

Cardiovascular diseases (CVD) are a major cause of morbidity and fatality across the world. Coronary plaque progression and fragile plaque characteristics have been associated with CVD. Several risk factors linked to CVD are smoking, diabetes, age, rheumatoid arthritis, obesity, HDL, LDL, cholesterol and hypertension etc. Along with environmental factors, many genetic factors contribute towards the onset and progression of this disease. For genetic information moving from DNA to protein, RNA serves as an unavoidable connecting connection. Epitranscriptomics has been proposed to play an important role in controlling different functions of RNA to various physiological processes of a cell. The most predominant modification in eukaryotic cells is m^{6}A which is regulated by a number of regulated proteins named generally as readers, writers and erasers. Single nucleotide polymorphism (SNP) in the promoter region is known to be involved in altering affinity for transcription factors resulting in variable gene expression. Keeping in view the importance of genetic variation in the form of SNPs, the present study was conducted to dissect the role A>C polymorphism in the promoter region of m6A writer gene *KIAA1429 (*rs3133659) and its correlation with CVD. In this study, blood samples were taken from hospitals of Rawalpindi and Islamabad, DNA was extracted. Polymorphism was studied through tetra-primer ARMS-PCR. Biochemical analysis from cases and controls shows a significant association of cholesterol, HDL, LDL and SBP in CVD ($p<0.05$). Comparison of genotypic and allelic frequencies using chi-square test z-test and odds ratio was done by Pearson and fischer model respectively. Results of the statistical analysis demonstrated that there were significant differences ($p<0.05$) in frequency distribution among cases and control. These results reflect that the *rs3133659 A>,* polymorphism in *KIAA1429* correlates with CVD. Findings of this study are needed to be verified further with a large sample size to explore the role of this polymorphism in the aetiology of CVD in the population of Pakistan.

Keywords: Cardiovascular disease (CVD), Single nucleotide polymorphism (SNP), N6-methyladenosine (m6A).

Introduction

The human heart is the most sophisticated designed structure of the body. There is no other structure in the human body like the heart. The heart is the most important functional system which is also self- regulated. The important mission of science is to do research on a particular disease and invent its medication. From some past years, research and advancement in the structure and function of the heart at cellular, genetic and molecular levels have opened a ground for new knowledge about diseases and clinical medicines related to heart.Heart failure is one of the commonest causes of death globally [1]. Heart failure is the biggest killer of the human population [2]. With coronary artery (CAD) and hypertension as the main causes of CVDs like angina, arrhythmias, atherosclerosis, and

Copyright: © 2026 The Author(s). Licensed under a Creative Commons Attribution (CC BY) 4.0 International License. Users are free to share and adapt the material by giving an appropriate credit to the original author(s).

myocardial infarction [3]. Genetic factors, consumption of high amounts of salt and alcohol, obesity, aging, and lack of insulin resistance are linked to hypertension. The first-line treatment is lifestyle changes, which are augmented with pharmacological intervention in case of required necessity as in many CVD, including CAD, hypertension is considered as a predisposing factor [4].

Cholesterol, calcium, fat, and inflammatory substances are accumulated in the walls of the vessels, a condition called atherosclerosis, which is frequently generated by oxidized phospholipids and platelet adhesion and lipoproteins [5]. Heart attack or myocardial infarction arises due to the disruption of the atherosclerotic plaques that contribute to the formation of blood clots that obstruct the coronary arteries resulting in ischemia [6].The presence of advanced plaques in the coronary arteries is a symptom of the CAD that causes significant predisposition to heart-related diseases and may be detected through coronary angiography [7]. Systolic heart failure, which comprises the left ventricular remodeling and dilatation, is a primary cause of hospitalizations and deaths of industrialized countries [8].Atherosclerosis is signified by endothelial dysfunction, which leads to plaque progression and predisposition [9,10,11]. The susceptibility of significant cardiovascular complications, cerebrovascular negative events, thrombotic events and congestive heart failure has increased [12]. Endothelial dysfunction is present in more than 50% of the angina and non-obstructive CAD patients [4] and this phenomenon can also be relevant to other organs as there is a systemic involvement of vascular endothelial dysfunction [13].

Cardiovascular disease is the number one killer worldwide because it is the one responsible for taking away a life after every 39 seconds compared to cancer [14]. CVD affects 82 million in the US, and 811,940 were killed in the year 2010-2011, respectively [7]. It will be estimated that up to 43.9% of Americans will have CVD in 2030. The problem is widespread in Pakistan because one of every four middle-aged adults is affected, and prevalence is increasing due to lack of physical activity, family history and sedentary lifestyles [15]. Diabetes is found to increase the risk of the disease by a factor of two up to three in atherosclerosis, mainly among females [16]. The previous studies demonstrated that the sources of CVD are hypertension, hypercholesterolemia, dysglycemia, LV hypertrophy, inflammation, obesity, smoking, and aging [17,18] . Breathlessness, fluid retention, and fatigue are symptoms of heart failure that are caused by cellular and molecular dysfunction and inflammatory cytokines such as TNF-alpha [19,20].

The mortality rate of CVDs has been minimized as a result of the development of diagnostic and surgical procedures, yet it is still significant [21]. One of the strategies is affordable medication and community-based measures which are effective at targeting the endothelial cells, myofibroblasts and cardiac myocytes. These active and home-based fitness programs can mitigate the risk of CVD and reverse damage caused by inactivity, which becomes more evident during the COVID-19 pandemics [22,23].

Genetic factors such as family history are contributors to the risk of CAD. Genome-wide association studies (GWAS) have exposed genetic variants that alter lipid metabolism and blood pressure and may be a therapeutic option [24]. N6-methyladenosine (m6A) RNA methylation, a form of epitranscriptomic regulation, influences gene expression, cell cycle and the progression of cardiovascular diseases. m6A modification is mediated by the writer protein KIAA1429, which is a major m6A-methyltransferase that controls mRNA splicing, mRNA stability, and the processing of mRNA [25,26]. The m6A changes happen to be associated with cardiac hypertrophy, heart failure, and vascular diseases [27,28] .The aim of this research was to analyze genetic variations in the promoter region of *KIAA1429* in

selected samples of the human population and to establish correlation, if any, between selected SNPs in promoter region of writer (*KIAA 1429*) with CVD.

Materials and methods

A case-control study was conducted at COMSATS University Islamabad and affiliated hospitals in Rawalpindi and Islamabad over a period of six months. The sample size was 100 consisting of 50 cases (patients diagnosed with CVD) and 50 controls (age and gender matched controls). Inclusion criteria were adults above the age of 30 years of either gender, of any ethnic background in Pakistan, with or without family history of CVD. Chronic diseases other than CVD were excluded, pregnant women, and those who do not consent were excluded. Potential confounding was reduced by matching case and control groups by age and gender.

Data collection

Structured questionnaires were acquired to get demographic and clinical variables. Recorded variables were age, gender, weight, height, body mass index (BMI), family, history of CVD, age at the onset of the disease, and the treatment regime. A detailed performa with personal and medical history was filled in by all the participants.

Sample collection

The collection of blood samples was performed with the help of a medical expert that confirms participants as diseased or normal on the basis of specific parameters. Sterile venous blood (5 mL) was drawn on each participant, under aseptic conditions. 2.5 mL of blood was collected into serum separator tubes with clot activators, and after centrifugation at 4000 rpm and 5 minutes, serum was aliquoted at -20 ^{0}C to cater for the evaluation of uric acid, triglycerides, cholesterol, HDL, and LDL. To extract DNA, 2.5 mL of blood was withdrawn into EDTA tubes and inverted to allow mixing with the anticoagulant before it was stored at 4 °C until use.

DNA extraction

Blood in EDTA was purified to obtain genomic DNA following the non-enzymatic salting-out procedure. The initial incubation of blood samples was done at 25 °C room temperature for 10 to 15 minutes. Solutions used for DNA extraction are as follows:

- 10mM Tris-HCl with pH 7.6, 10mM KCl, 10mM MgCl2, 2Mm EDTA
- TKM2: 10m Tris-HCl pH7.6, 10mM MgCl2 , 2mM EDTA, 10mM KCl 0.4 M NACl,
- TKM1 Triton:98 ml TKM1,2ml Triton
- 10%SDS 10 gm of SDS in 100 ml of distilled water.
- Saturated NaCl 6M
- 70% of Ethanol 70 ml of the absolute ethanol in 30 ml of distilled water. TE (DNA dissolving 10mM Tris-HCl, 1mM EDTA, pH 8 Buffer)
- TKM1-triton buffer was used to lyse the cells, and their nuclear pellets were washed using TKM1. Pellets were dissolved in the TKM2 buffer with SDS to lyse nuclear membranes. The precipitation of proteins was performed in saturated NaCl and DNA

in absolute ethanol. DNA pellets were washed, dissolved in TE buffer, and kept at 4 °C until further analysis.

- In the process of DNA extraction, nucleic acid stability and purity were achieved using specific reagents. **Ethylene DiaamineTetra Acetic Acid** (**EDTA):** It is a chelating agent chelate with cations like Mg+2 and Ca+2 and used to hinder the DNase activity, enzyme which lyses the DNA. The chelator EDTA blocks the cofactor Mg+2 and Ca+2 by binding their sites.
- Tris HCL: Genomic DNA is pH sensitive; the Tris HCl is useful in maintaining pH by interacting with lipopolysaccharides which are present in cell membranes to allow permeabilization.
- Sodium dodecyl sulphate (SDS): SDS is the anionic cleansing agent that aids in breakup of nuclear envelope and cell membrane.
- Saturated NaCl: As Na+ ion makes an ionic bond with the negative charge of DNA neutralizes DNA to protect from denaturation. For isolation of the protein from DNA a large amount of NaCl is added. DNA or RNA can be precipitated from the solution containing high percentage of ethanol.so DNA precipitated out of solution the process is commonly referred as “Salting Out”.
- KCL: Use of KCL salt in solution helps to neutralizes the positive charge on the proteins and DNA. As a result of neutralization, it will not be able to form strong ionic bonds thus can easily be separated.
- $MGCL_2$: it keeps the DNA save and defends DNA by mixing with other organelles of the cell as after lysis of the cell, there is no compartment left in a cell. The MgCl2 interferes with the negative charge of lipoproteins of cell membrane.
- 70%ethanol: 70% of ethanol added and was mixed gently to remove any excess salts.
- Absolute ethanol: Ethanol has much lower dielectric constant, compared to water as it promotes the aggregation of DNA. DNA is not soluble in the alcohol layer so it will be precipitated out.
- TE Buffer: TE butter is used to prevent the DNA for degreation DNases were inhibited by EDTA chelating divalent cations and pH was stabilized using Tris-HCl. SDS permeabilized cell membranes, released DNA and NaCl/KCl helped to remove/stabilize proteins. During lysis, MgCl2 spared DNA. Precipitation and recovery of the nucleic acids were possible using Ethanol and TE buffer was utilised in the dissolution and storing over a long time.

Quality assessment and genotyping

The quality of DNA was assessed under 1% agarose gel stained with ethidium bromide. The genotyping of SNPs in the KIAA1429 promoter was done by ARMS-PCR, employing two outer and two inner primers (allele specific). ARMS PCR mainly depends on mismatch strategy. This technique relies on 3'terminal nucleotides of PCR that should be allele specific. Subsequently DNA polymerase is only capable for primer extension when its 3’ end is totally complementary to the template giving rise to PCR amplicon. DNA genotyping is done basically to confirm whether the amplicon is produced or not. The PCR conditions were: initial denaturation of 95 °C, 5 min.; 35 cycles of 95 °C, 1 min.; 55 °C, 1 min., extended to 72 °C, 1 min.; and extension of 72 °C, 10 min. PCR products were electrophoresed on 3% agarose gels containing 100 bp ladders. An internal control was applied in tetra-primer ARMS-PCR to simultaneously differentiate between mutant and normal alleles.

Amplification-refractory mutation system (ARMS) PCR is a simple, financially sustainable and convenient method for identifying SNP. In this technique we used 4 primers in one PCR followed by gel electrophoresis. In the current investigation, SNPs in the core promoter region of kIAA1429 gene were genotyped by ARMS PCR method.

Visualization of amplified product

The amplicon was analyzed on 3% agarose gel electrophoresis; 5µl of PCR product mixed with 2µl loading dye was loaded into the wells of the gel. One of the wells contained 2µl of 100 bp DNA ladder (mostly the central well). Gel electrophoresis was performed for 50 min at 90 volt and 500 mA followed by visualized under Ultraviolet light using the trans-illuminator, sizes of the bands that appeared on the gel were studied in comparison with the sizes present in the ladder and the images of the gels were saved in the gel documentation system (Alpha-Imager Mini Bucher Biotech, Basel, Switzerland).

Tetra primer ARMS-PCR analysis

Amplification refractory mutation system (ARMS)-PCR, as allele-specific oligonucleotide PCR is a procedure basically formulated to discover the known sequence polymorphisms. In ARMS- PCR, 2 primer pairs in a single PCR tube can concurrently amplify both normal alleles and mutant allele and it also permits amplification of an internal DNA control. Each outer primer combines with the that of opposite inner primer to produce smaller allele-specific amplicons, which are of various sizes and can easily be separated on gel electrophoresis either as homozygous or heterozygous conditions of PCR. KIAA1429 PCR thermal cycles included an initial denaturation of 95 degree Celsius, 5 min and 35 cycles of denaturation, annealing, and extension. The cycles were 1 minute denaturation at 95 °C, 1 minute annealing at 55 °C, and one minute extension at 55 °C. Cycling was followed by a final extension at 72 °C in 10 min to complete the amplification of the target product. So, the PCR products were isolated on 3% agarose gel and the gel was viewed using the Gel documentation system. Amplified PCR products showed three fragment sizes of 500 bp, 321bp and 220 bp sizes respectively.

Ethical approval

Ethical approval of this research project was obtained from the Ethical committee of COMSATS University Islamabad and hospitals of Rawalpindi and Islamabad from where the samples of CVD were collected (IRB Approval no. 210/COMSATS Islamabad). Aims and objectives for this study were properly explained to participants through Informed consents.

Statistical analysis

Data representation was done in the form of means, standard deviation, frequencies and percentages. Allele and genotype frequencies were calculated by direct counting method. Chi- square ($\chi 2$) and Fischer's exact test was performed to compare distribution of allele and genotype frequencies between case and control groups, where needed. Odds ratios (ORs) with 95% confidence intervals (95% CI) were calculated to estimate the risk presented by a particular allele.

Results

The current study was conducted to analyse the association of *KIAA1429* A>C SNP (rs3133659) in CVD patients of Pakistani population. A total of 128 subjects were investigated which includes 76 CVD patients and 50 control subjects.

Demographic and biochemical characteristics of CVD patients

The present study comprises 78 CVD patients (40 males and 38 females) having mean age of 51.35 years and 50 controls (50 males and 50 females) with mean age of 50.38 years as shown in table 1. Significant results for cholesterol, HDL (high density lipoproteins), LDL (low density lipoproteins), TG (triglycerides) and SBP (systolic blood pressure) are observed in CVD patients while age, BMI (body mass index), D.B.P (diastolic blood pressure) shows non- significant results as mentioned in the table 1 and figures shows relative frequencies.

Table 1: Demographic and Biochemical characteristics of study population

Parameters	**Patients (n=78)**	**Controls (n=50)**	**p Value**
Age (Years)	51.35 ± 14.02	50.38 ± 8.56	0.66
BMI (kg/m2)	27.30 ± 4.46	27.11 ± 2.54	0.7
Cholesterol (mg/dL)	182.11 ± 55.31	119.4 ± 35.64	<0.0001
HDL (mg/dL)	33.86 ± 10.83	38.36 ± 10.06	0.01
LDL (mg/dL)	149.25 ± 23.32	87.78 ± 19.17	<0.001
TG (mg/dL)	121.93 ± 64.84	88.28 ± 24.99	0.0006
S.B.P (mm Hg)	139.55 ± 19.44	125.08 ± 8.54	<0.0001
D.B.P (mm Hg)	86.53 ± 10.13	85 ± 6.70	0.34

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure TG:Triglycerides, LDL: Low density lipoproteins HDL: High density lipoproteins, P values were calculated by Pearson chi-square test

Frequency percentage analysis

The relative frequencies of age, BMI, SBP and DBP, cholesterol, TG, LDL and HDL of CVD and controls are shown in figures. The lower and upper limit of age was 16 – 80 years respectively in CVD (Figure 3.1) while in controls it is 40 – 77 (figure 2) respectively. 16.5 to 42.9 kg/m^2 are the lower to upper limit of BMI in CVD (Figure 3). While it is 23-34 kg/m^2 for the controls group (Figure 4). In CVD the lower to upper limit of cholesterol was 75-361 mg/dL (Figure 5) and in controls it is 59-196 (Figure 6). The lower to upper value for HDL in CVD patients was 3.2-106 mg/dL (Figure 7) while in controls it is 25 to 76 (Figure 8). The lowest to highest value of low-density lipoproteins (LDL) was 80 mg/dL to 213mg/dL in patients (Figure 9) and in controls it is 43 to 121 (Figure 10). In CVD 27-228 mg/dL was lower to higher value of triglycerides (TG) (Figure 11) and in controls it is 43 and 144 respectively (Figure 12). Bell shaped distribution was seen Systolic blood pressure was varying among CVD patients having lowest to highest value 100-190 mmHg (Figure 13) and in controls it is 110-150 mmHg (Figure 14). The lower to upper value of diastolic blood pressure was 70-110 mmHg in CVD (Figure 15) and 80-100mmHg in controls (Figure 16).

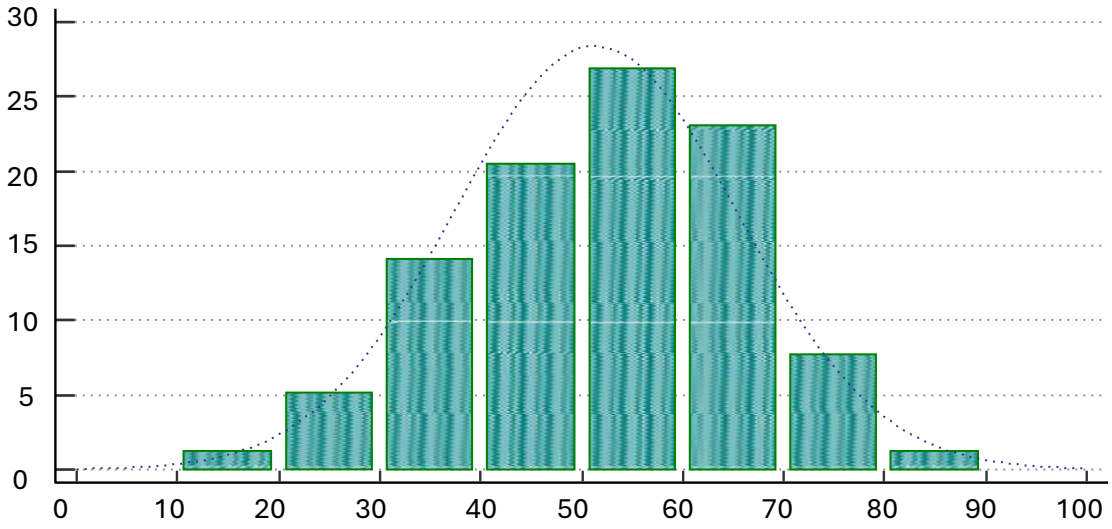


Figure 1: Age of CVD Patients.

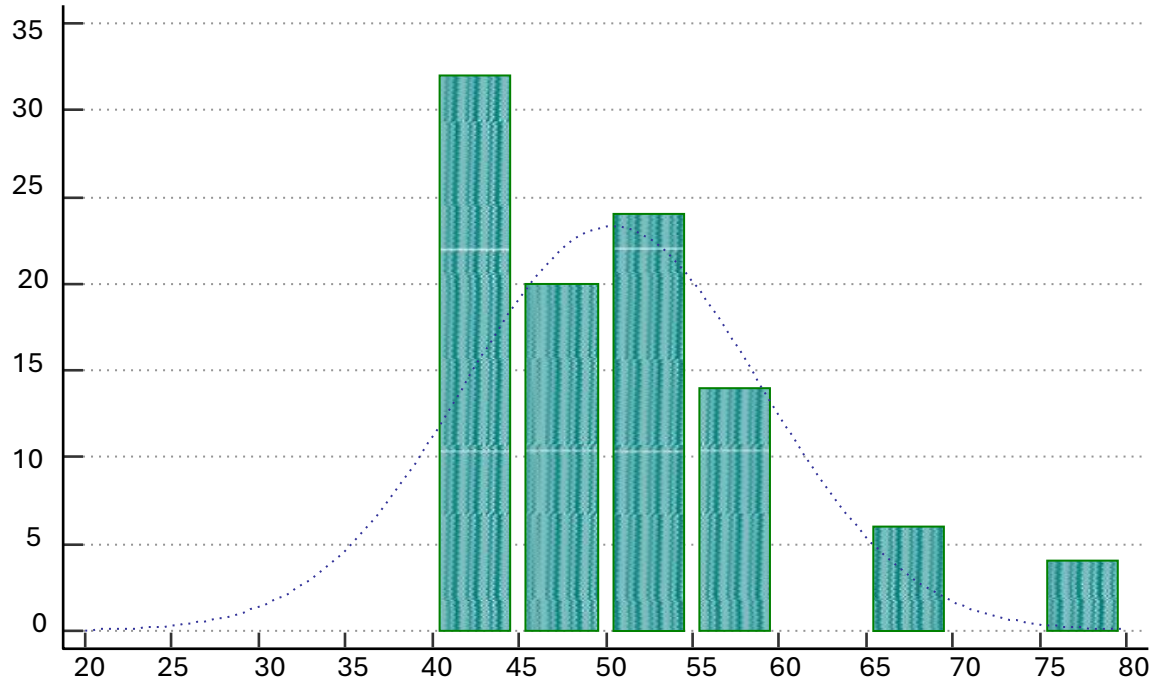


Figure 2: Age of control

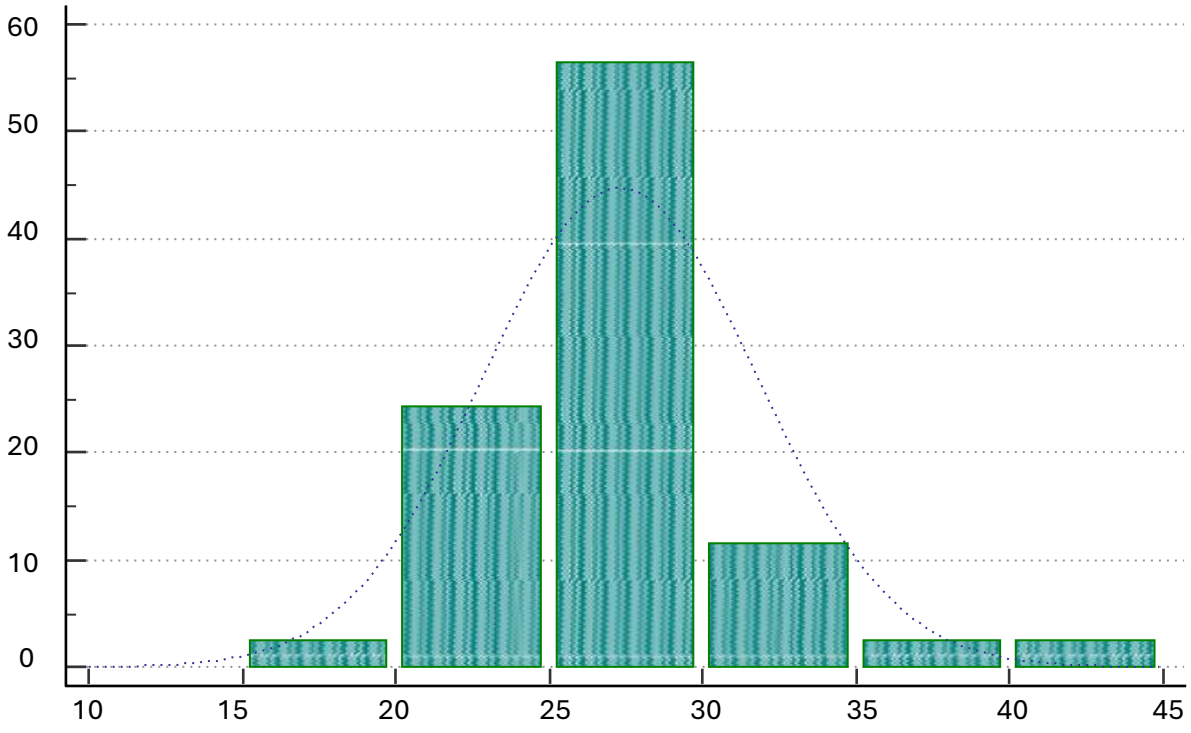


Figure 3: BMI of CVD patients.

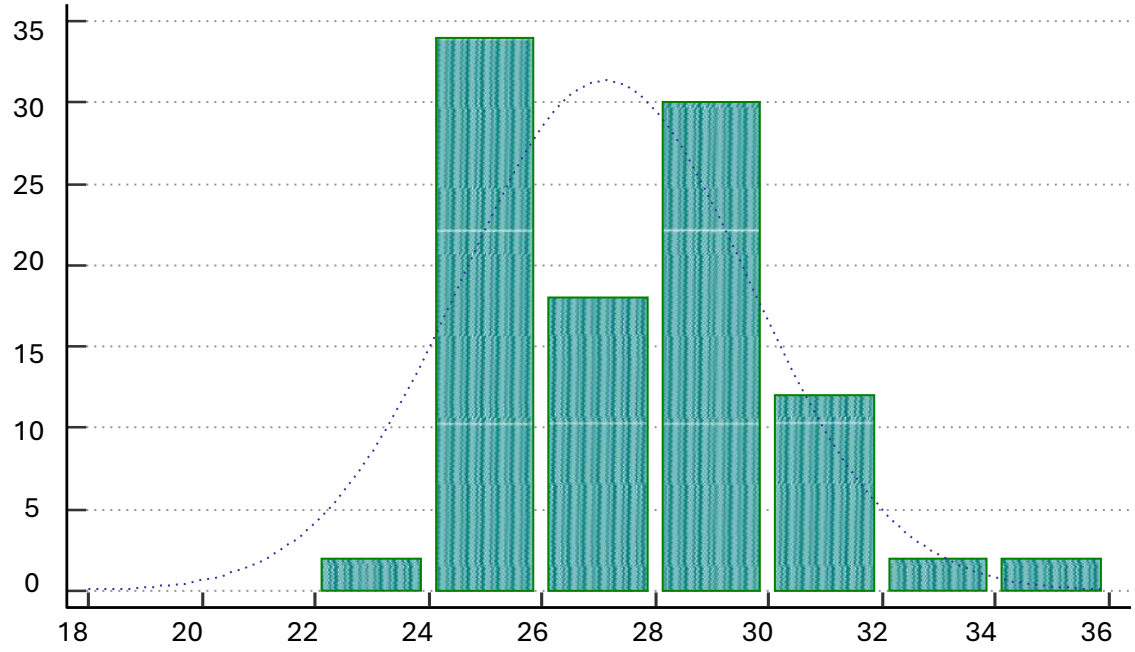


Figure 4: Age of controls group

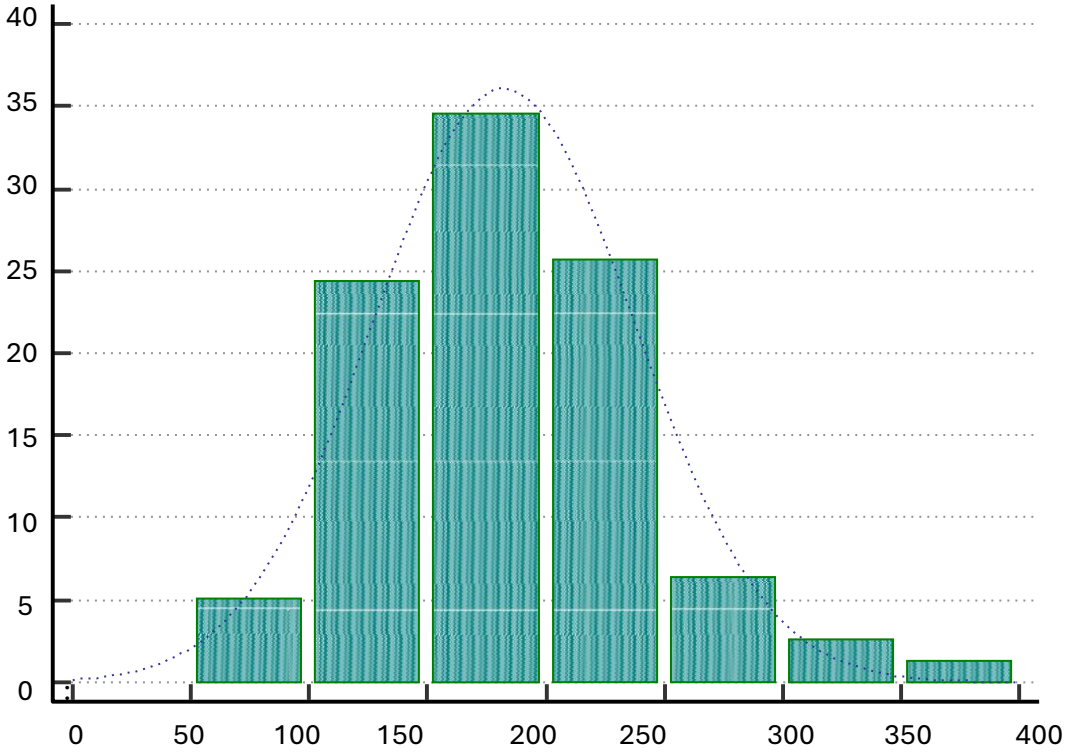


Figure 5: Cholesterol level of CVD patients

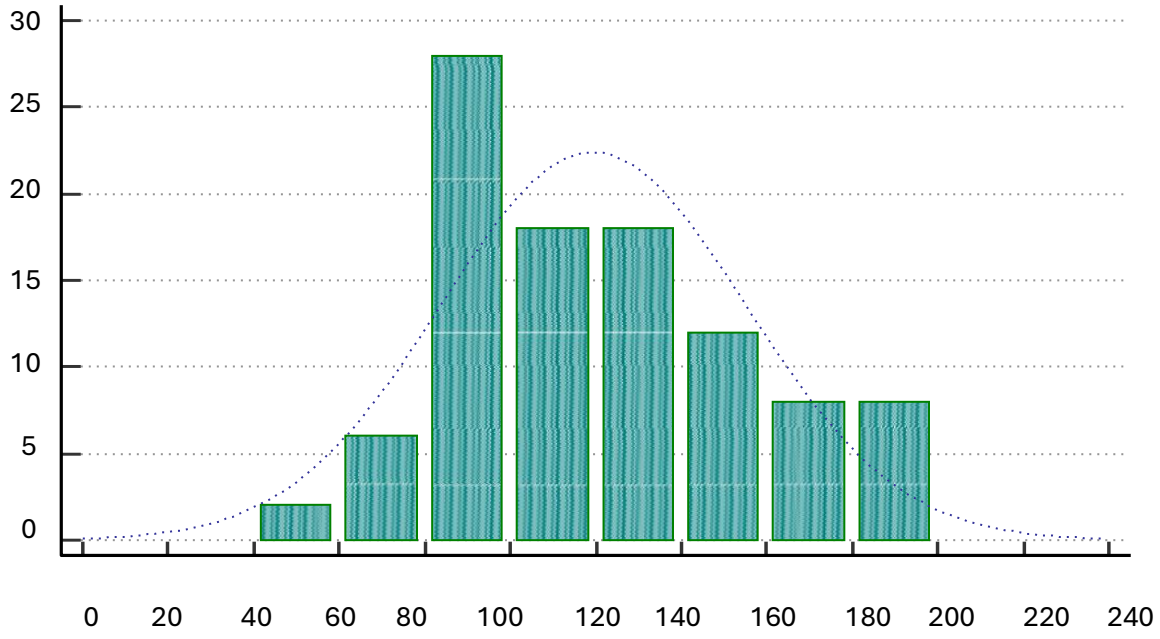


Figure 6: Cholesterol level of controls

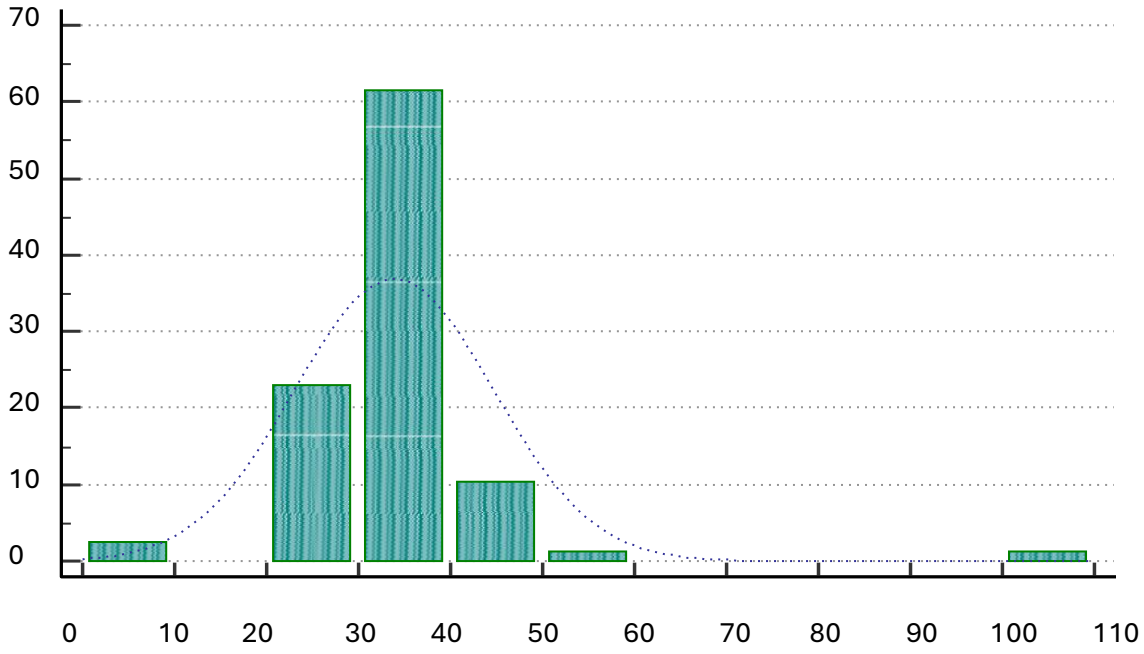


Figure 7: HDL of patients

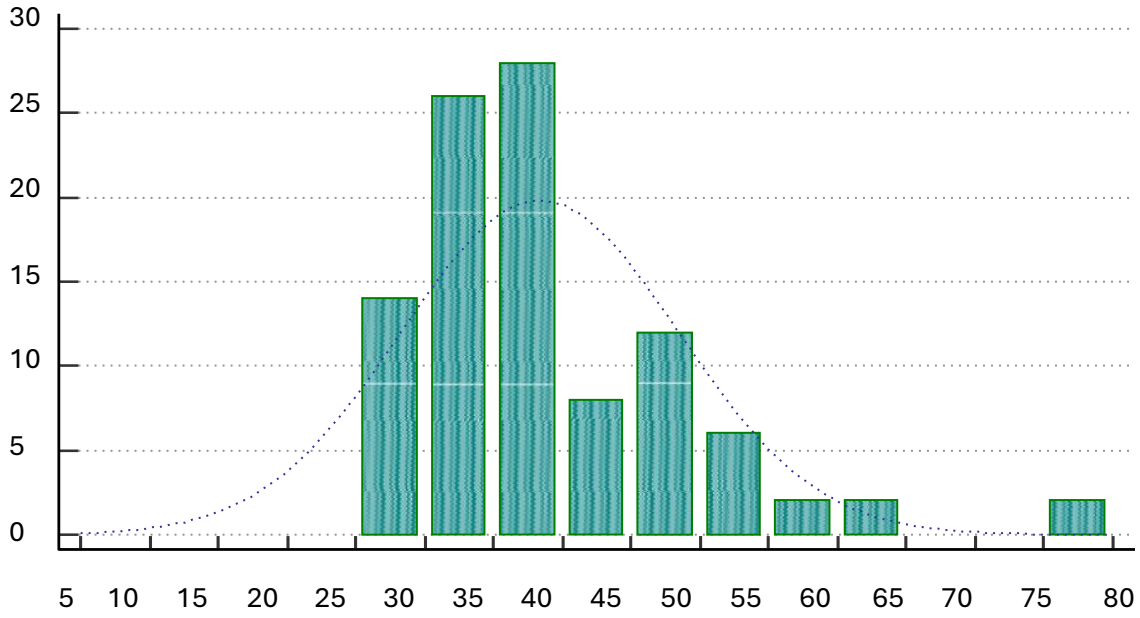


Figure 8: HDL of controls.

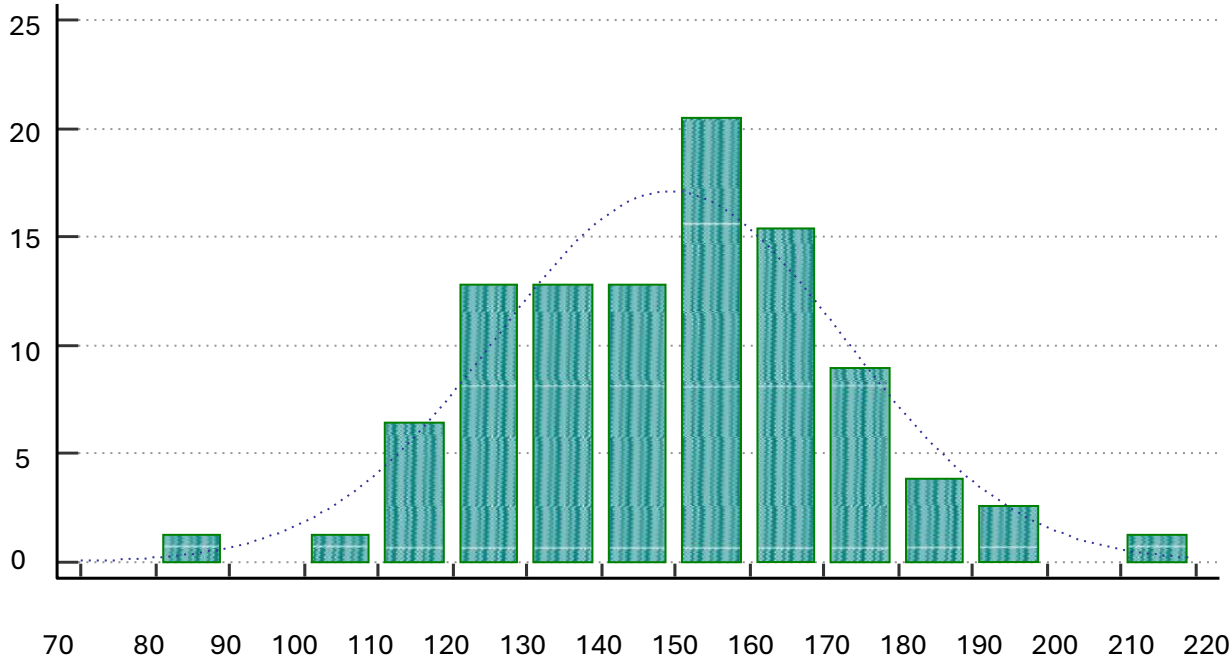


Figure 9: LDL of CVD patients

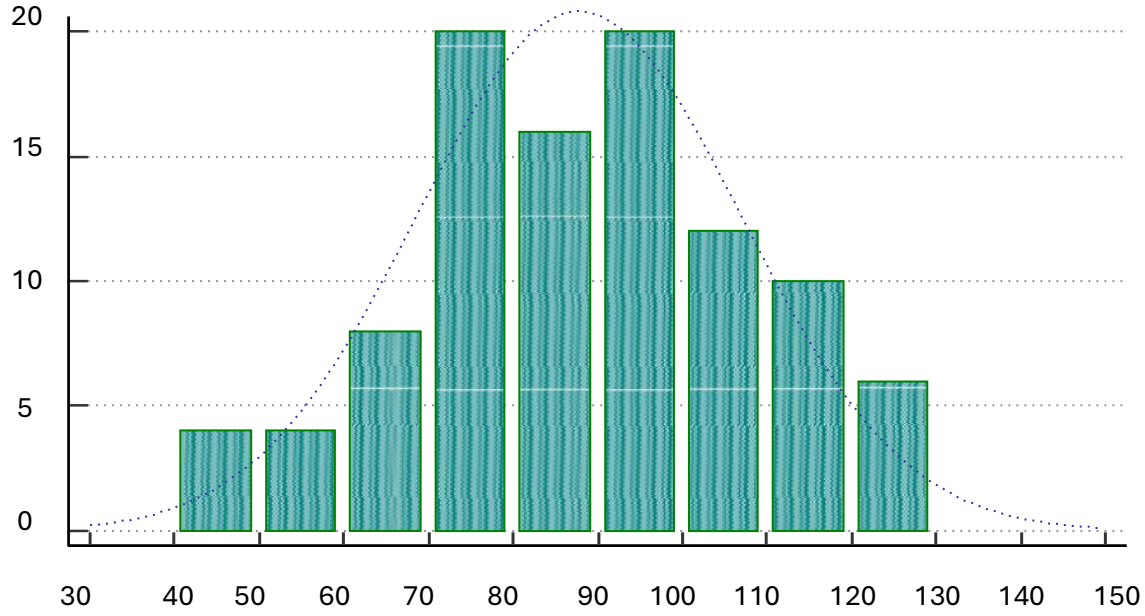


Figure 10: LDL of controls.

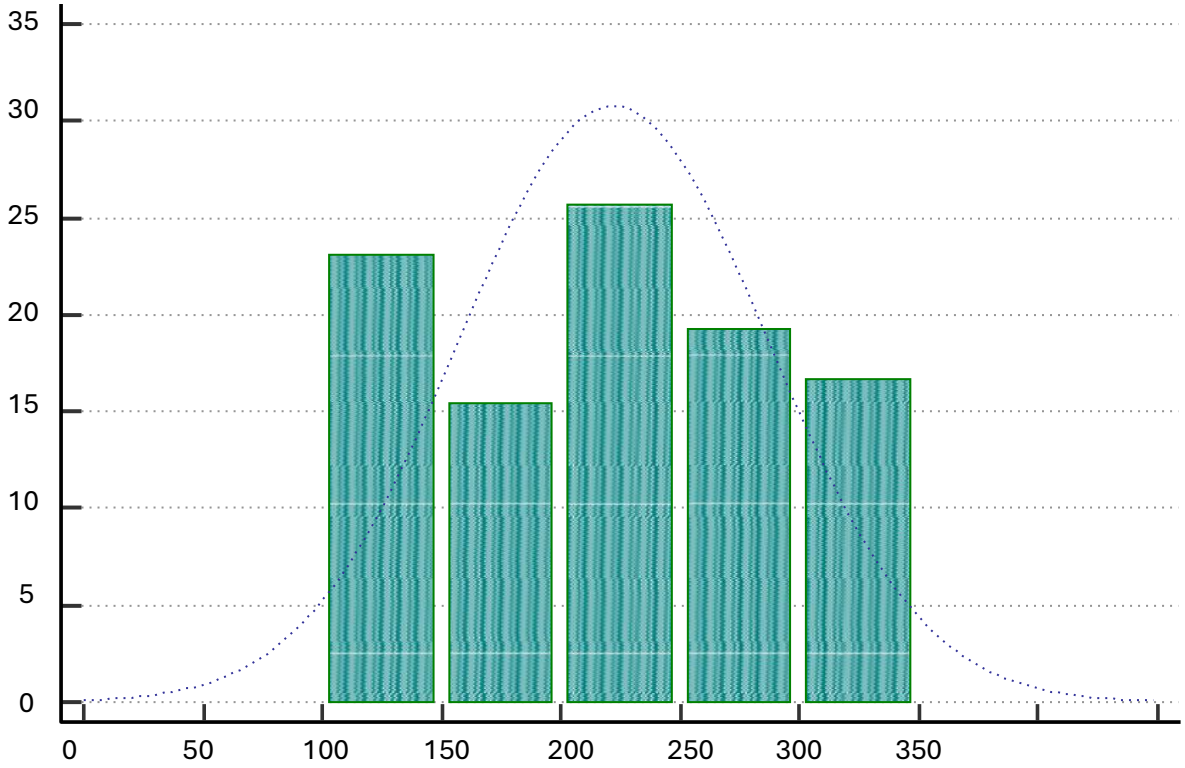


Figure 11: TG of CVD patients.

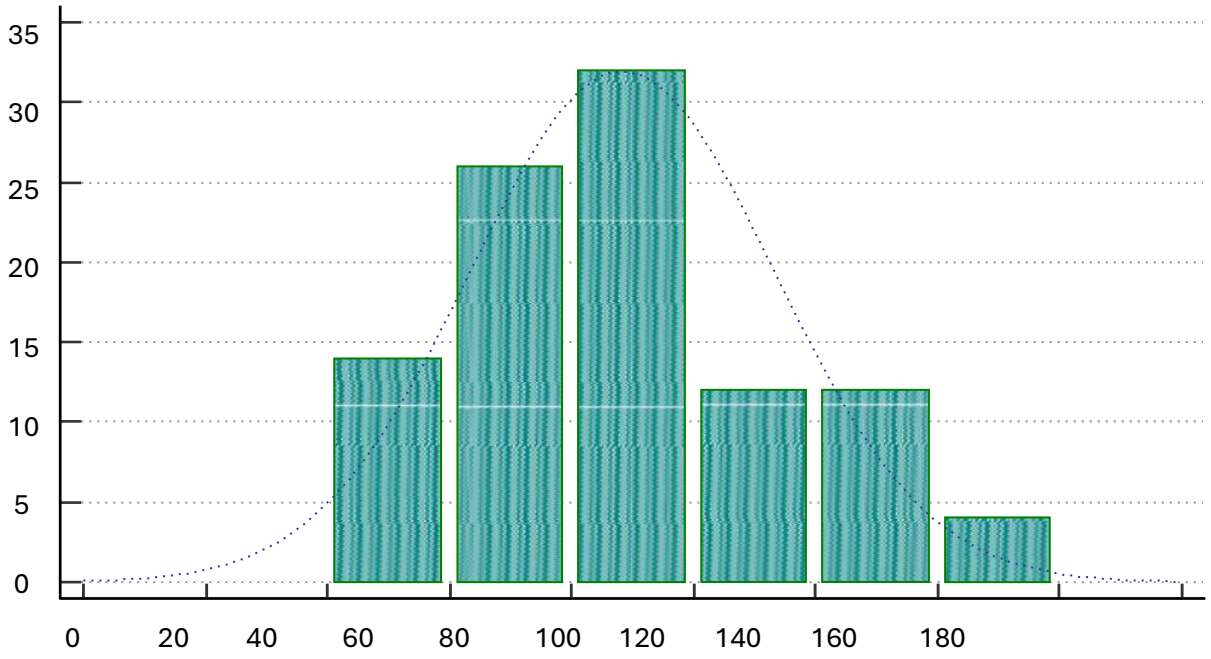


Figure 12: TG of controls.

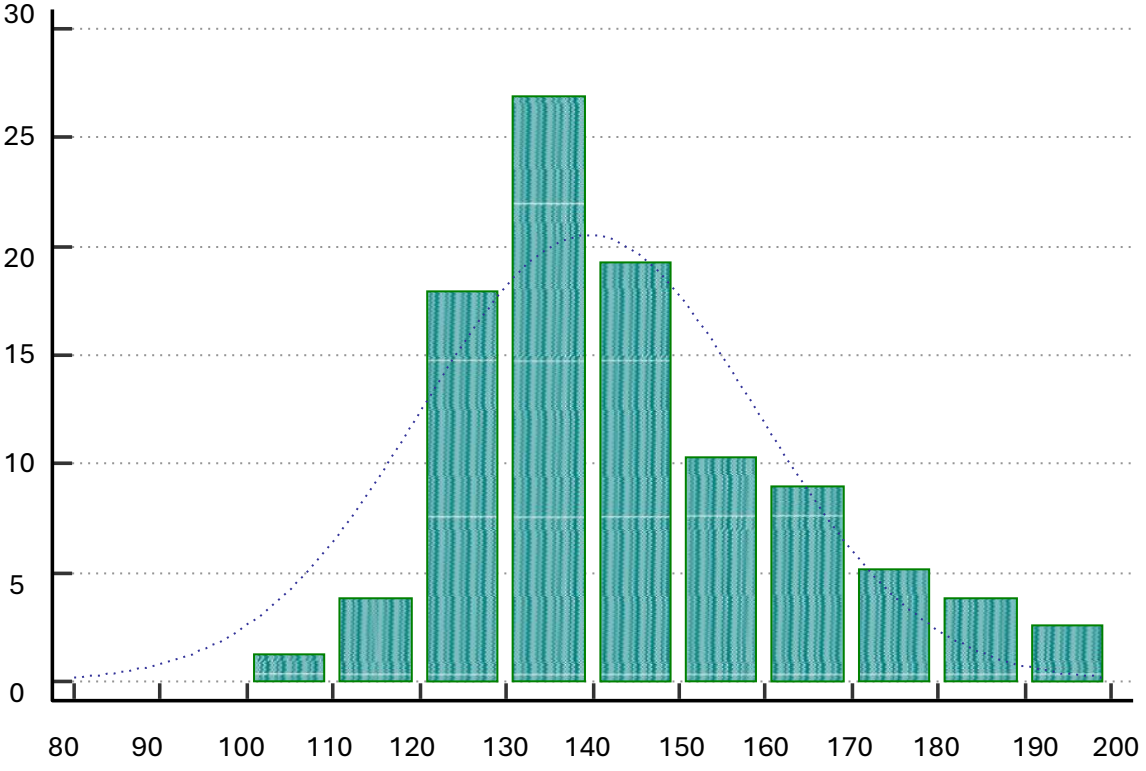


Figure 13: SBP of CVD patients.

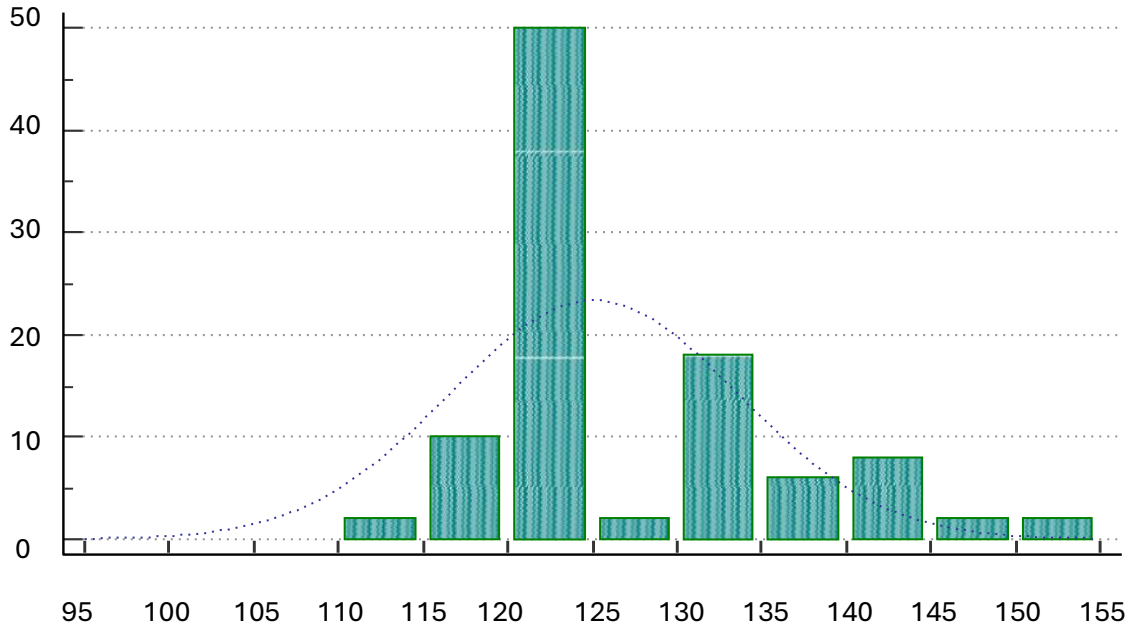


Figure 14: SBP of controls.

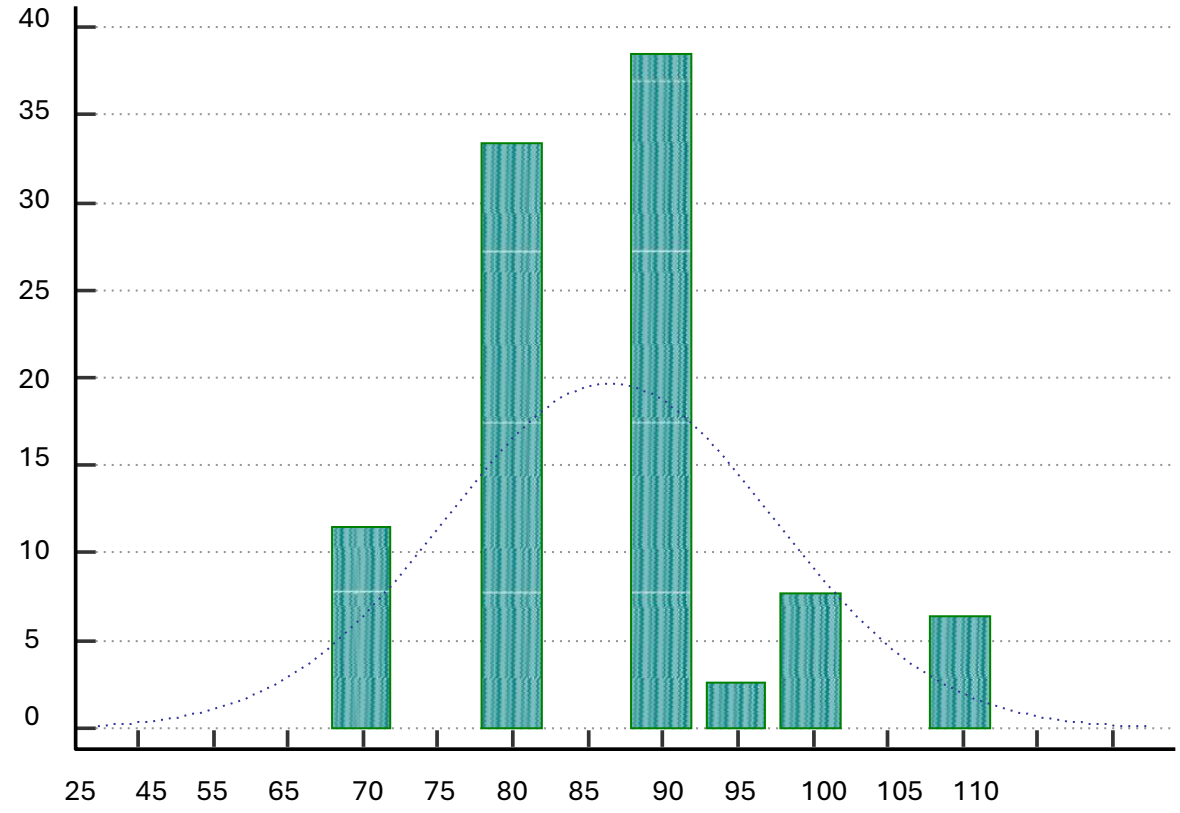


Figure 15: DBP of CVD patients.

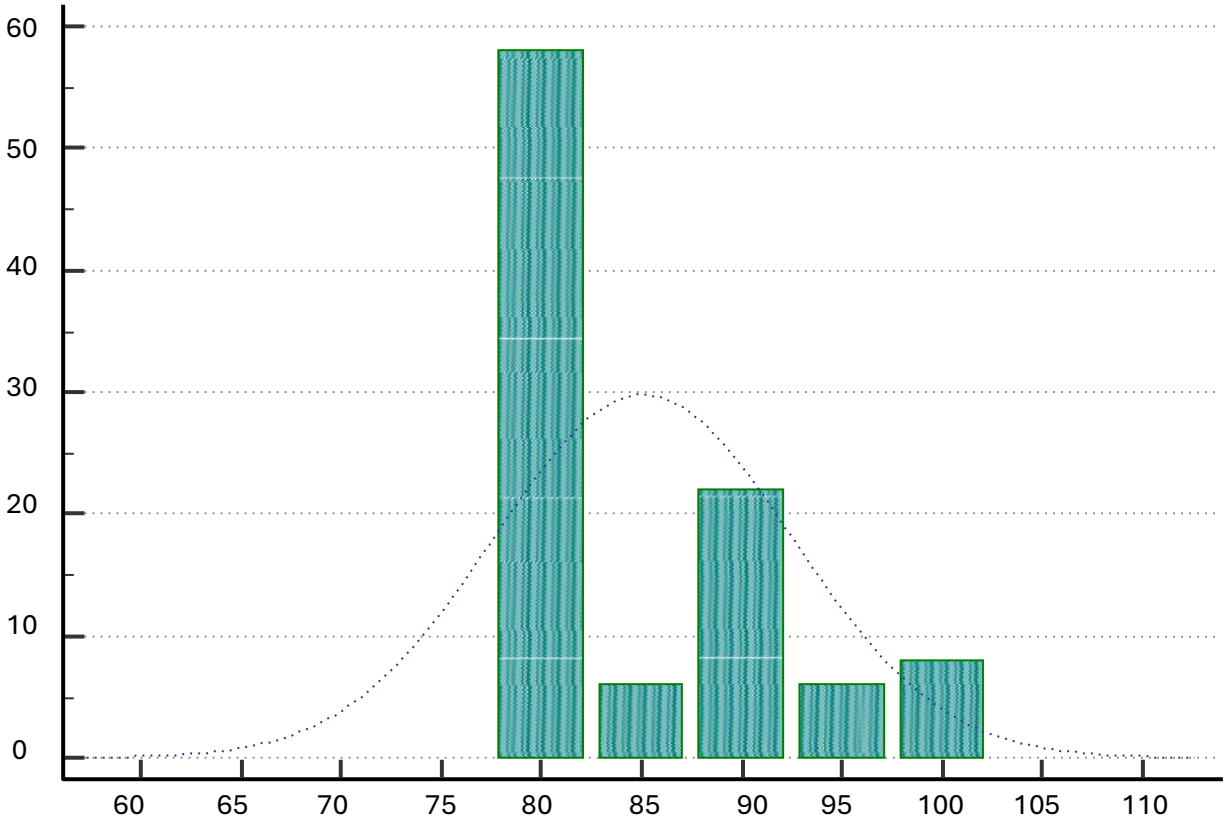
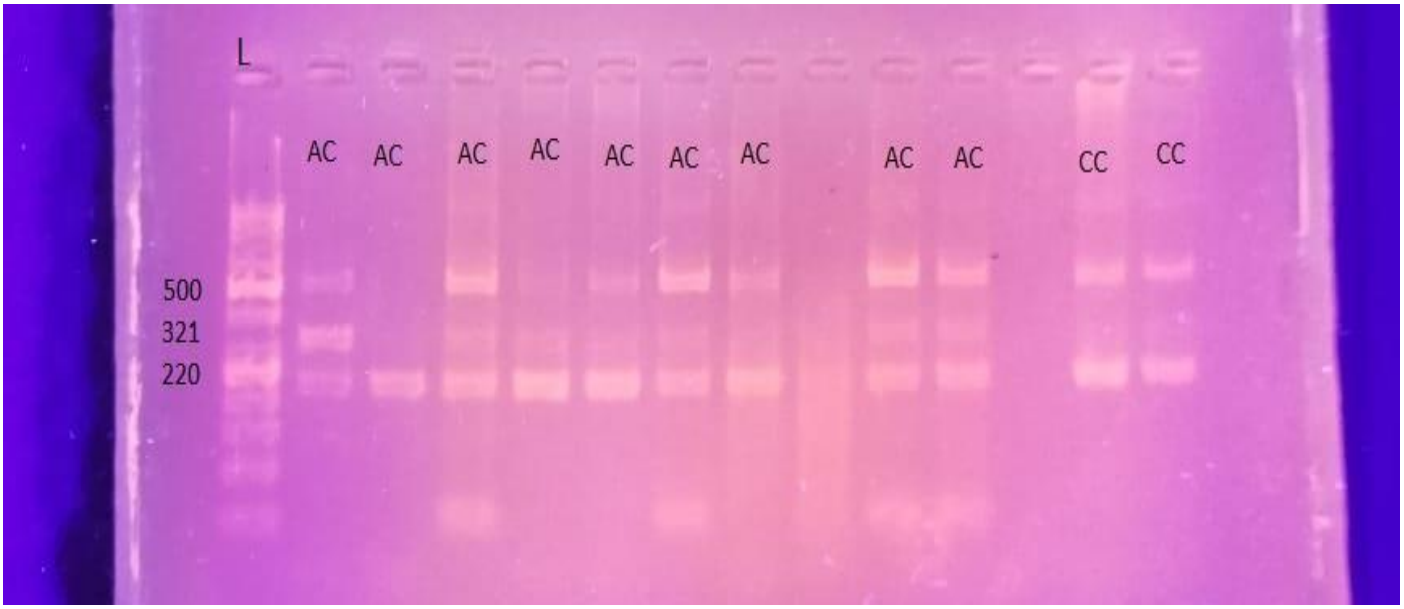


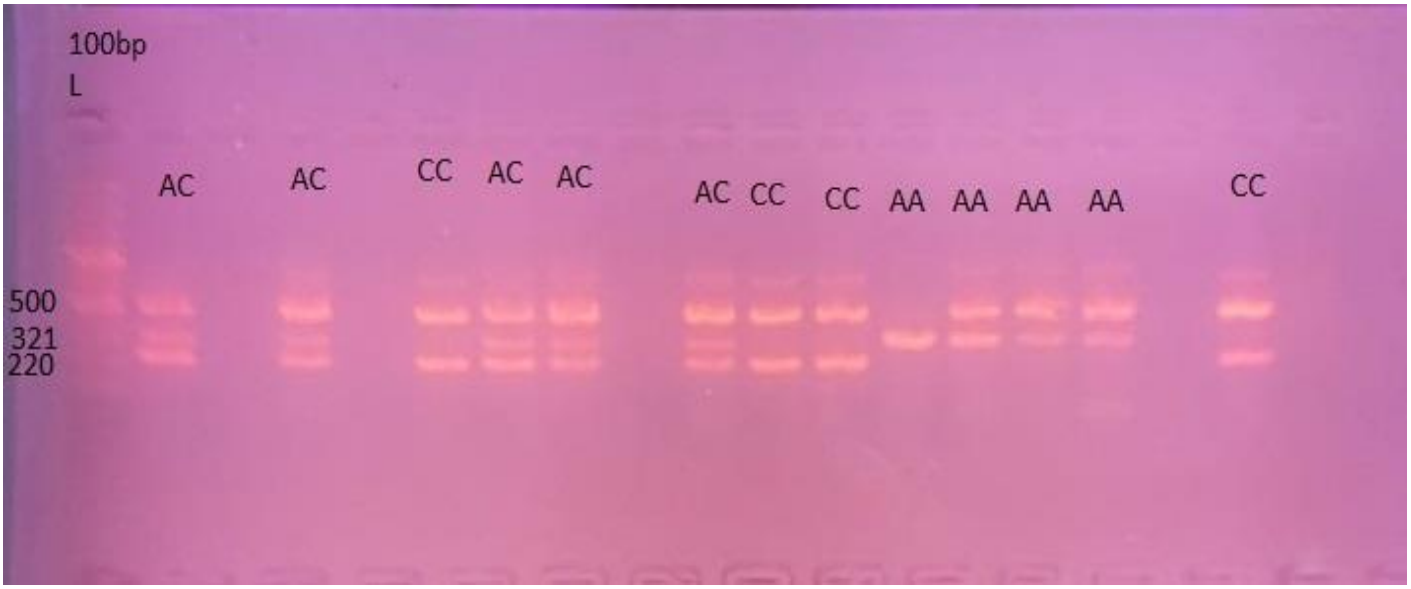
Figure 16: DBP of controls.

Genotyping and allele frequency analysis

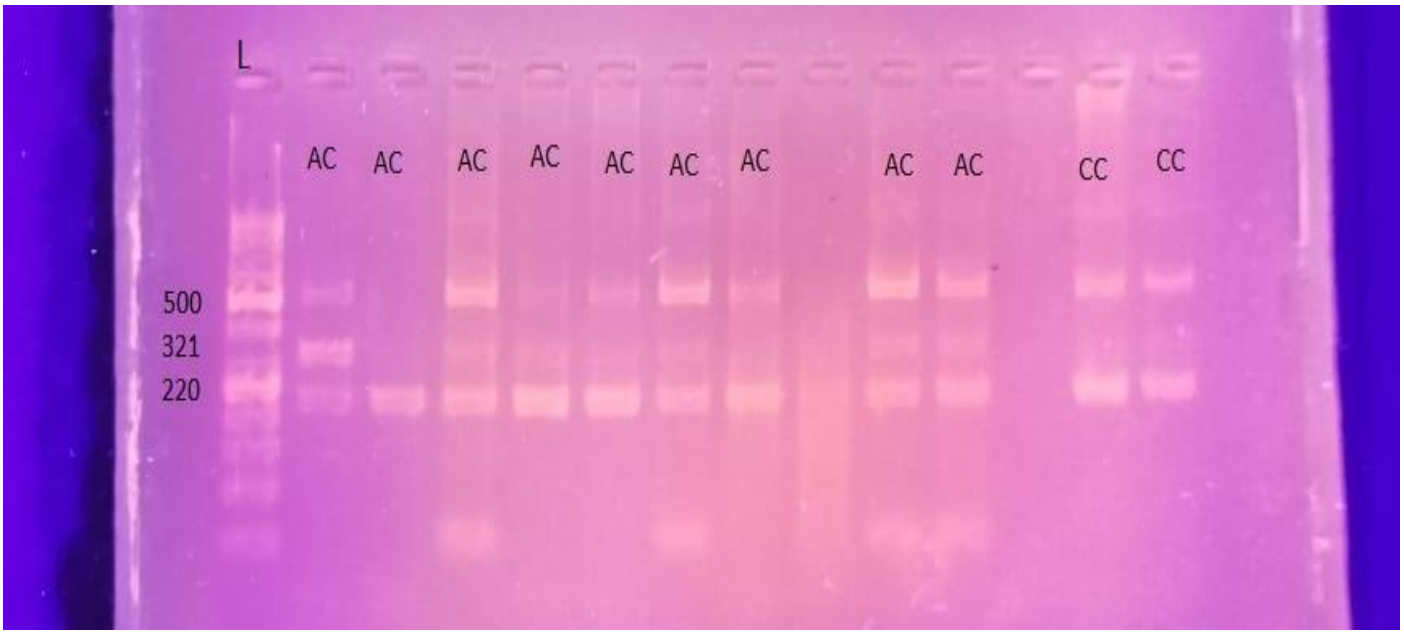
A total of 128 subjects, including 78 CVD patients and 50 control subjects were assessed for the association of Kiaa1429 polymorphism with CVD. Three different bands of 500bp, 320 bp and 221 bp were determined by using 3% agarose gel with 50 and 100bp ladders shown in figure 17. The AC genotypes were 73% in CVD and 44% in controls. The important findings CC were 23% in CVD and 12% in controls. AA genotypes were observed as 3.8% in patients and 44% in controls. (Table 2). Significant association was noted for the genotypes among the patients and controls. (chi = 31.32, P <0.05). The dominant modal for KIAA1429 polymorphism also elaborates the significance association of CC+AC in CVD and controls (chi = 31.25, P <0.05: Table 2). Moreover, the frequency of A allele was 40% in CVD and 66% in controls. While the frequency of C allele in CVD and controls were 60% and 34 % respectively. The frequencies of A and C alleles among the CVD and controls indicated significant association with p<0.05.


L
AC AC AC AC AC AC AC AC AC CC CC
500
321
220

a)


100bp
L
AC AC CC AC AC AC CC CC AA AA AA AA CC
500
321
220

b)


L
AC AC AC AC AC AC AC AC AC CC CC
500
321
220

c)

Figure 17(a), (b), (c): KIAA1429 PCR products

Table 2: The genotype and allele frequency of KIAA 1429 A>C in study population

SNP	CVD patients (n=78)	controls (n=50)	χ2 (P value)
A>C Polymorphism			
AC	57 (73%)	22 (44%)	
CC	18 (23%)	6 (12%)	
AA	3 (3.8%)	22 (44%)	31.32 (p<0.00001)a
AA	3 (3.8%)	22(44%)	31.25 (p<0.0001)a
CC+AC	75 (96.2%)	28 (56%)	
A allele	63(40%)	66 (66%)	(p=0.00004) [b]
C allele	93(60%)	34 (34%)	OR= 0.349 95%C.I= 0.2069- 0.5887

Values are given as number and percentage, P[a] values calculate by pearson's chi-square test, P[b] values calculate by Fisher exact test.

Discussion

CVD is the third major leading cause of morbidity and fatality across the world. Many predisposing factors are related with the development of disease such as age, obesity, LDL, HDL and TG etc [29]. In a current literature, it was observed that excessive gain in the BMI from childhood onwards is associated with CVD while in our sample population analysis of 78 cases and 50 controls shows no significant association of BMI as a risk factor to CVD (p=0.7). However, another study held in 2020 in the Mediterranean population suggested that clinical and genetic analysis showed that TG, HDL and cholesterol was associated with CVD events however LDL in these studies showed that it was not associated with disease events [29]. While in the current study on the Pakistani population our sample population shows significant association of cholesterol, TG, HDL and LDL (p<0.05) as risk factors towards the pathogenesis of CVD. Genetics analysis in recent years suggest several genetic mutations that play a key role in developing CVD such as MED13, MED23 and MED25 have been associated with heart malformations in humans.

RNA modifications play a major role in gene regulation and expression. In recent decades >100 types of RNA mutations have been discovered. These modifications are controlling the physiological state of a cell for a biological process to proceed accurately. m6A (N6 -methyladenosine) is the most abundant and reversible methylation induced. m6A is present in tRNA, rRNA and viral RNA in a variety of organisms including viruses, yeast and mammals [30,31]. 25% of the transcriptome carries m6A marks for expression regulation. It is significantly involved in mRNA stability, splicing, nuclear translocation and translation processes [31]. Methyltransferases (writers-*METTL3*, *METTL14*, *WTAP*, *KIAA1429* etc.), demethylases (erasers-*FTO*, *ALKBH5* etc.) and readers (*YTHDF1, YTHDF2, YTHDC1* etc.) has a crucial part in dynamic regulation of m6A modification [32].

CVD is linked with dysregulation of RNA modification being induced by any kind of epigenetic modification or genetic variants. SNP is the most common type of genetic variant, responsible for genetic variation and diversity. SNPs can alter promoters thus regulating gene expression. Recent work on m6A SNPs have revealed their association with many diseases e.g. cardiovascular disease [33]. Understanding the role of RNA modifications in CVD may lead towards a better understanding of disease mechanisms and the development of novel biomarkers or therapeutic strategies [33].

Recent research showed that m6A modification is closely related to the pathogenesis of CVDs, such as heart failure, cardiac hypertrophy, ischemic heart disease, vascular calcification, pulmonary hypertension, aortic aneurysm, etc. Here in, the recent progress in m6A modification in CVD is briefly reviewed [34].

The aim of this study was to analyse association of m6A writer *KIAA1429* Gene SNP (rs3133659) A>C for CVD in Pakistani population. Till now, *KIAA1429* polymorphism has not been extensively studied for their disease association especially in Pakistan. Therefore, it was of prime importance to check whether the genetic variant in *KIAA1429* confers the risk of CVD or not.

KIAA1429 SNP primers were optimized at 55oC and samples which included both control and disease were screened. Appearance of bands at 320 or 221 bp regions shows the homozygosity while appearance of 3 bands shows heterozygosity. Statistical Analysis was done in three steps. Three models were designed such as Genotype frequency model, Allelic frequency model, and variant frequency model. Results are significant when p-value is less than 0.05. There were significant differences found between CVD and controls in all three models of *KIAA1429* Gene SNP (rs3133659). Chi-square values were calculated by Pearson and odd ratios by fisher model.

The genotype data of *KIAA1429* have revealed that the genotype frequencies of AC,CC and AA were 73%, 23% and 3.8% respectively in the disease group and genotype frequencies of AC,CC and AA was 44 % , 12% and 44 % respectively in the control group. Significant differences were observed among patients and control groups with p value less than 0.05. There was also a significant difference in the allelic frequencies in both groups. Apparently these results indicate that there is a strong association of *KIAA1429 with* CVD. Higher incidence of C allele in AC + CC genotype was found in CVD patients (3.8%, 96%) compared to healthy controls (44%, 56% respectively) indicating the risk in CVD patients (OR=0.349, 95%CI= 0.2069- 0, p= 0.0004), but still more research is required by increasing sample size, clinically evaluated controls as well as better genotyping methods may help in generating improved results.

A limitation of the current study is the relatively small and unequal sample size between the patient group (n=78) and the control group (n=50), which may limit the statistical power to detect rare allelic variants."

Conclusion

On the whole, this is the first study of screening of *KIAA1429* gene SNP (rs3133659) polymorphism in a Pakistani population of 78 cases and 50 controls, and a significant association with susceptibility to CVD has been evaluated. The findings of the present investigations support that *KIAA1429* Gene SNP (rs3133659) might has a role in pathogenesis of CVD. To verify and validate the role of the said and other genetic variants of *KIAA1429* gene, further studies involving larger sample sizes from Pakistani and other populations is needed.

Conflict of interest

The author declares no conflict of interest.

Funding

The author received no any financial support for this publication.

References

1. Shah AM, Claggett B, Sweitzer NK, Shah SJ, Anand IS, O'Meara E, et al. Cardiac structure and function and prognosis in heart failure with preserved ejection fraction: findings from the echocardiographic study of the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) Trial. *Circ Heart Fail*. 2014;7(5):740–751. https://doi.org/10.1161/CIRCHEARTFAILURE.114.001583
2. Shah AM, Shah SJ, Anand IS, Sweitzer NK, O'Meara E, Heitner JF, et al. Cardiac structure and function in heart failure with preserved ejection fraction: baseline findings from the echocardiographic study of the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist trial. *Circ Heart Fail*. 2014;7(1):104–115. https://doi.org/10.1161/CIRCHEARTFAILURE.113.000887
3. Carretero OA, Oparil S. Essential hypertension. Part I: definition and etiology. *Circulation*. 2000;101(3):329–335. https://doi.org/10.1161/01.cir.101.3.329
4. Sara JDS, Corban MT, Prasad M, Prasad A, Gulati R, Lerman LO, et al. Prevalence of myocardial bridging associated with coronary endothelial dysfunction in patients with chest pain and non-obstructive coronary artery disease. *EuroIntervention*. 2020;15(14):1262–1268. https://doi.org/10.4244/EIJ-D-18-00923
5. Brito V, Mellal K, Zoccal KF, Soto Y, Ménard L, Sarduy R, et al. Atheroregressive Potential of the Treatment with a Chimeric Monoclonal Antibody against Sulfated Glycosaminoglycans on Pre-existing Lesions in Apolipoprotein E-Deficient Mice. *Front Pharmacol*. 2017;8:782. https://doi.org/10.3389/fphar.2017.00782
6. Massberg S, Brand K, Grüner S, Page S, Müller E, Müller I, et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. *J Exp Med*. 2002;196(7):887–896. https://doi.org/10.1084/jem.20012044
7. Ruiz-Esparza GU, Flores-Arredondo JH, Segura-Ibarra V, Torre-Amione G, Ferrari M, Blanco E, et al. The physiology of cardiovascular disease and innovative liposomal platforms for therapy. *Int J Nanomedicine*. 2013;8:629–640. https://doi.org/10.2147/IJN.S34241
8. Thum T, Galuppo P, Wolf C, Fiedler J, Kneitz S, van Laake LW, et al. MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. *Circulation*. 2007;116(3):258–267. https://doi.org/10.1161/CIRCULATIONAHA.107.690248
9. Gimbrone MA. Vascular endothelium: an integrator of pathophysiologic stimuli in atherosclerosis. *Am J Cardiol*. 1995;75(6):67B-70B. https://doi.org/10.1016/0002-9149(95)80016-h
10. Yoon MH, Reriani M, Mario G, Rihal C, Gulati R, Lennon R, et al. Long-term endothelin receptor antagonism attenuates coronary plaque progression in patients with early atherosclerosis. *Int J Cardiol*. 2013;168(2):1316–1321. https://doi.org/10.1016/j.ijcard.2012.12.016
11. Lovell MJ, Mathur A. Cardiac stem cell therapy: progress from the bench to bedside. *Heart*. 2010;96(19):1531–1537. https://doi.org/10.1136/hrt.2009.190678
12. Borlaug BA, Olson TP, Lam CSP, Flood KS, Lerman A, Johnson BD, et al. Global cardiovascular reserve dysfunction in heart failure with preserved ejection fraction. *J Am Coll Cardiol*. 2010;56(11):845–854. https://doi.org/10.1016/j.jacc.2010.03.077
13. Corban MT, Lerman LO, Lerman A. Endothelial Dysfunction: Cardiovascular Disease Pathophysiology Hidden in Plain Sight. *Arterioscler Thromb Vasc Biol*. 2019;39(7):1272–1274. https://doi.org/10.1161/ATVBAHA.119.312844
14. Forman D, Wenger NK. What do the recent American Heart Association/American College of Cardiology Foundation Clinical Practice Guidelines tell us about the evolving management of

coronary heart disease in older adults? *J Geriatr Cardiol*. 2013;10(2):123–128. https://doi.org/10.3969/j.issn.1671-5411.2013.02.002
15. Jafar TH, Jafary FH, Jessani S, Chaturvedi N. Heart disease epidemic in Pakistan: women and men at equal risk. *Am Heart J*. 2005;150(2):221–226. https://doi.org/10.1016/j.ahj.2004.09.025
16. Zhang Y, Liu B, Zhao R, Zhang S, Yu XY, Li Y. The Influence of Sex on Cardiac Physiology and Cardiovascular Diseases. *J Cardiovasc Transl Res*. 2020;13(1):3–13. https://doi.org/10.1007/s12265-019-09943-6
17. Saleheen D, Zhao W, Rasheed A. Epidemiology and public health policy of tobacco use and cardiovascular disorders in low- and middle-income countries. *Arterioscler Thromb Vasc Biol*. 2014;34(9):1811–1819. https://doi.org/10.1161/ATVBAHA.114.303109
18. Litwin SE, Adams TD, Davidson LE, McKinlay R, Simper SC, Ranson L, et al. Longitudinal Changes in Cardiac Structure and Function in Severe Obesity: 11-Year Follow-Up in the Utah Obesity Study. *J Am Heart Assoc*. 2020;9(12):e014542. https://doi.org/10.1161/JAHA.119.014542
19. Heart Failure Society of America, Lindenfeld J, Albert NM, Boehmer JP, Collins SP, Ezekowitz JA, et al. HFSA 2010 Comprehensive Heart Failure Practice Guideline. *J Card Fail*. 2010;16(6):e1-194. https://doi.org/10.1016/j.cardfail.2010.04.004
20. Coletta AP, Clark AL, Banarjee P, Cleland JGF. Clinical trials update: RENEWAL (RENAISSANCE and RECOVER) and ATTACH. *Eur J Heart Fail*. 2002;4(4):559–561. https://doi.org/10.1016/s1388-9842(02)00130-9
21. Joyner MJ. Effect of exercise on arterial compliance. *Circulation*. 2000;102(11):1214–1215. https://doi.org/10.1161/01.cir.102.11.1214
22. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT, et al. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet*. 2012;380(9838):219–229. https://doi.org/10.1016/S0140-6736(12)61031-9
23. Safiyari-Hafizi H, Taunton J, Ignaszewski A, Warburton DER. The Health Benefits of a 12-Week Home-Based Interval Training Cardiac Rehabilitation Program in Patients With Heart Failure. *Can J Cardiol*. 2016;32(4):561–567. https://doi.org/10.1016/j.cjca.2016.01.031
24. Kessler T, Vilne B, Schunkert H. The impact of genome-wide association studies on the pathophysiology and therapy of cardiovascular disease. *EMBO Mol Med*. 2016;8(7):688–701. https://doi.org/10.15252/emmm.201505833
25. Cheng X, Li M, Rao X, Zhang W, Li X, Wang L, et al. KIAA1429 regulates the migration and invasion of hepatocellular carcinoma by altering m6A modification of ID2 mRNA. *Onco Targets Ther*. 2019;12:3421–3428. https://doi.org/10.2147/OTT.S202865
26. Hu Y, Ouyang Z, Sui X, Qi M, Li M, He Y, et al. Oocyte competence is maintained by m6A methyltransferase KIAA1429-mediated RNA metabolism during mouse follicular development. *Cell Death Differ*. 2020;27(8):2468–2483. https://doi.org/10.1038/s41418-020-0515-1
27. Batista PJ, Molinie B, Wang J, Qu K, Zhang J, Li L, et al. m(6)A RNA modification controls cell fate transition in mammalian embryonic stem cells. *Cell Stem Cell*. 2014;15(6):707–719. https://doi.org/10.1016/j.stem.2014.09.019
28. Stellos K. The rise of epitranscriptomic era: implications for cardiovascular disease. *Cardiovasc Res*. 2017;113(5):e2–e3. https://doi.org/10.1093/cvr/cvx041
29. Castañer O, Pintó X, Subirana I, Amor AJ, Ros E, Hernáez Á, et al. Remnant Cholesterol, Not LDL Cholesterol, Is Associated With Incident Cardiovascular Disease. *J Am Coll Cardiol*. 2020;76(23):2712–2724. https://doi.org/10.1016/j.jacc.2020.10.008
30. Roignant JY, Soller M. m6A in mRNA: An Ancient Mechanism for Fine-Tuning Gene Expression. *Trends Genet*. 2017;33(6):380–390. https://doi.org/10.1016/j.tig.2017.03.007
31. Schöller E, Weichmann F, Treiber T, Ringle S, Treiber N, Flatley A, et al. Interactions, localization, and phosphorylation of the m6A generating METTL3-METTL14-WTAP complex. *RNA*. 2018;24(4):499–512. https://doi.org/10.1261/rna.064394.117

32. Schwartz S, Mumbach MR, Jovanovic M, Wang T, Maciag K, Bushkin GG, et al. Perturbation of m6A writers reveals two distinct classes of mRNA methylation at internal and 5' sites. *Cell Rep*. 2014;8(1):284–296. https://doi.org/10.1016/j.celrep.2014.05.048
33. Dorn LE, Lasman L, Chen J, Xu X, Hund TJ, Medvedovic M, et al. The N6-Methyladenosine mRNA Methylase METTL3 Controls Cardiac Homeostasis and Hypertrophy. *Circulation*. 2019;139(4):533–545. https://doi.org/10.1161/CIRCULATIONAHA.118.036146
34. Qin Y, Li L, Luo E, Hou J, Yan G, Wang D, et al. Role of m6A RNA methylation in cardiovascular disease (Review). *Int J Mol Med*. 2020;46(6):1958–1972. https://doi.org/10.3892/ijmm.2020.4746

Publisher's Note: *The views, data, claims, and opinions expressed in this article are solely those of the authors and do not necessarily reflect the official policy or position or endorsement of the publisher, editors, reviewers, or their affiliated organizations. No legal liability is assumed by the publisher, editors, or reviewers for the accuracy or practical use of the data disclosed. The publication of this material does not make the publisher, editorial team, or reviewers responsible for any damages incurred due to the information described in this article.*