DOI: https://doi.org/10.53350/pjmhs2023174338

ORIGINAL ARTICLE

Prevalence and Association of LPA Gene Variant the Statin Therapeutic Response in Cardiovascular Patients

ZAKIA SHAFQAT[1], SHAGUFTA NAZ[2], MUHAMMAD AKRAM TARIQ[3]
[1]*Department of Zoology, Lahore College for Women University Lahore, Pakistan*
[2]*Assistant Professor, Department of Zoology, Lahore College for Women University Lahore, Pakistan*
[3]*Professor, Punjab Higher Education Department Lahore, Pakistan.*
Correspondence to: Zakia Shafqat, Email: Zakiashafqat17@gmail.com, Cell: +923351149745

ABSTRACT

Purpose: Cardiac patients who fail to achieve critical biochemical parameters are considered as resistant. Differences in drug absorption, transport, in trapeutic drug metabolism, drug metabolism in other organs, and drug excretion mechanisms can all be linked to tatin resistance. Numerous single nucleotide polymorphisms (SNPs) have been discovered in the solute carrier organic transporter LPA gene, with three common SNPs labelled as 872G> A, 220 T>C and 384 A>G which are thought to be the most common and responsible for the LPA transporter's suitable transport function.

Method: Cardiac patients (Both Males and Females i.e. n=90) who were on statin therapy from last one year were engaged to evaluate the statin treatment response. DNA was extracted from blood samples of all cardiac patients in the study. To amplify the targeted region of SNPs of gene LPA, an allele specific PCR extension was performed. Allele specific extension based PCR products for three SNPs were electrophoresed on agarose gel to find out the possible variant in each patient. To validate gel-based identified genotypes, selected PCR products for possible allelic variants of each genetic marker were sequenced using the Sanger's Sequencing method.

Results: For SNP (rs104455872), the genotype GA (66.6%) was found to be the most frequent genotype in comparison to genotype AA (20%) and GG (13.3%) in this study group. Similarly, for genetic marker (SNP rs3798220) the genotype frequencies of genetic variant TC (70%), is higher as compared to other genotypes TT (13.30%), CC (16.6%). For genetic marker rs 74617384) AG was found higher in the genotype AG (83.3%) in comparison to other genotype GG (10%) to other minor allele. In overall cardiac patients, the rosuvastatin was prescribed to more cardiac patients (55.17%) than the atorvastatin (44.83%). The genotype GA (rs104455872) was more frequently prescribed rosuvastatin by the physician (36.3%) in comparison to atorvastatin (30%) for the same genotype GA. All other two genotypes of marker rs104455872 were least frequently prescribed with both statin salts (rosuvastatin & atorvastatin). Similarly, the genotype TC of marker, (rs3798220), was more frequently prescribed rosuvastatin by the physician (43.3%) in comparison to atorvastatin (26.6%) for the same genotype TC. The genotype TT of marker, rs3798220, found rarely prescribed by both statin salts i.e. rosuvastatin (0.0%) & atorvastatin (3.4%). The genotype AG (rs74617384) was more frequently prescribed rosuvastatin by the physician (46.6%) in comparison to atorvastatin (36.6%%) for the same genotype AG. All other three genotypes of marker rs2306283 were least frequently prescribed with both statin salts (rosuvastatin& atorvastatin).

Conclusions: The genotyping of cardiac patients for the genetic markers (rs 104455872), (rs3798220)or (rs 74617384)) of gene LPA gene may be helpful to tailor the statin salt for precision therapy and may reduce the adverse effects in cardiac patients.

Keywords: Single nucleotide polymorphism, lipoprotein particle A, Hydroxy methyl glutarate co enzyme A, Statin Associated Muscle Symptoms, LDL-C, HDL-C

INTRODUCTION

Cardiovascular disease is the major cause of mortality among teenagers and adults, worldwide. A number of factors that contribute to (CVDs), includes: alcohol drinking (Xi *et al.,* 2017) disturbed sleep patterns (Song *et al.*, 2019), stress, smoking and poor eating habits (Menotti *et al*., 2012).

Statin is a combination of seven different salts. The primary target organ for all statins is liver. The liver is responsible for regulating dyslipidemia and hypercholesterolemia in the bloodstream. Statins function by blocking (HMGCR) which is the enzyme responsible for limiting cholesterol synthesis (HMGCR). This enzyme is converted into precursor of cholesterol known as mevalonic acid. Enzymes that bind to their active sites change their conformational structure. In this way, it is prevented that HMG-CoA reductase develops an un-functional structure. These medicines are particularly effective and selective because of the conformational change at the active site.

The pharmacodynamics of statin medicines are governed by genes involved in cholesterol biosynthesis and lipoprotein metabolism. Moreover, number of DNA polymorphisms in these genes have been linked to inter-individual variance in statin response (Schachter *et al*., 2005). A highly atherogenic particle, lipoprotein (a), is encoded by the LPA gene. Single nucleotide polymorphism is the most prevalent type of genetic variation identified in humans (Consortium *et al*., 2010). 75 to 95 percent of lipoprotein (a) levels are heritable, and single-nucleotide variations in the LPA gene are the most common cause.

Statin efficacy is predicted by ABCG2, SLCO1B1, CYP3A4, HMGCR, and LPA. These genes have been independently linked to the response of LDL cholesterol to statin treatment. Seven SNPs within the LPA/PLG gene were related with coronary heart disease (CHD) occurrences when taking statins. An association between the rs10455872 SNP and the reduction of LDL cholesterol with multiple statins has been found in numerous clinical trials, as well as a meta-analysis of GWA studies (Postmus *et al.,* 2014). In the European population, rs10455872 at the LPA/PLG gene on chromosome 6 showed the strongest correlation. A study in Korea found that statins were ineffective for hypercholesterolemia patients.

Biobank database in UK identified genetic variants' simvastatin (rs10455872 & rs74617384) and atorvastatin (rs429358) which were strongly associated with the lipid and cholesterol levels. An intronic A>G SNP known as Rs10455872 has been related to a higher risk of cardiac disease in Europeans population (Li *et al.,* 2011). Simvastatin's LDL-lowering efficacy was favorably correlated with another LPA gene SNP (rs3798220) in the Heart Protection Study (Hopewell *et al*., 2013).

Statin toxicity or intolerance is mostly manifested as SAMSs (Akao *et al*., 2012). Statin adverse effects are mostly on muscles which make almost 72% of all statin toxicity myalgia myopathy Related (Backes *et al.,* 2017). Age, gender, ethnicity, frailty, genetics, the presence of other diseases, and polypharmacy are all risk factors. A deeper understanding of individual genotypes would be advantageous for both cardiac patients and the economy.

MATERIALS AND METHODS

Sampling: In accordance with ethical norms, blood samples from 90 cardiac patients on statin treatment were collected in EDTA vials from different hospitals in Lahore The experimental work was done in the Genetics lab of Lahore College for Women University and Surgimed laboratory, surgimed hospital Lahore. The patients'relatives gave their written consent forms.

Extraction of DNA: A DNA extraction kit was used to extract DNA from fresh blood samples of patients and healthy controls. However, an organic method of DNA extraction (33) was also used. .Using agarose gel electrophoresis, the quality and quantity of DNA was measured, as stated below.

Design of primers: For allelic variations of rs10455872, (rs3798220) or rs74617384) allele-specific amplification primers were created using Primer 3 (v. 0.4.0) software from the website, http://frodo.wi.mit.edu. With allele-specific primers, genomic DNA bordering the SNP was amplified. For SNP amplification, two distinct sets of primers were used: one wild-type allele-specific primer and the other mutant allele-specific primer. The universal primer was non-allele specific, and wild and mutant genotypes of each marker had the same primer. The allele-specific primers were designed according to the procedure (Hirotsu's et al 34. The optimal annealing temperatures for each variety, as well as the primer sequences.

Allele-specific polymerase chain reaction (PCR) amplification: In a 20µL. reaction volume, 10 ng genomic DNA template 3µl, 0.3 µl 0.15 µM of each oligonucleotide primer, 10µl 2X PCR Taq Plus Master mix with dye (Applied Biological Materials, Canada), and 6.4µl dH2O were used for PCR. The conditions set for the PCR amplification were as follows: The following PCR cycling conditions were carried out: 3 minutes at 95°C for 1 cycle, 35 cycles at 95°C for 30 seconds, with varied annealing temperatures 3.3 for 30 seconds and 72°C for 30 seconds followed by 1 cycle at 72°C for 5 minutes.

Agarose Gel Electrophoresis: Products of SNPs were replicated, electrophoresed, and examined with a UV trans illuminator on a 2 percent agarose gel stained with EtBr. The gel-based genotyping approach was used to genotype the LPA varients rs10455872, (rs3798220) or rs74617384)

Sanger Sequencing: To validate the gel-based methodology of SNP detection, Sanger sequencing of purified PCR products from chosen samples was performed to confirm the unique allelic variants of the LPA gene. The amplification of PCR involves the following steps of pre-denaturation at 95˚C for 2 minute, followed by 36 cycles of denaturing at 97˚C for 20 seconds, annealing at 56˚C for 15 seconds, and extension at 70˚C for 5 minute, and a final extension at 50˚C for 6 minute. The sequencing was performed on an ABI PRISM 3100Automated sequencer (Applied Bio systems). The sequencing results were assembled using ABI PRISM sequencing analysis software version 3.7 (Applied Bio systems) and chromatograms were analyzed with Chromas software (www.technelysium.com.au/chromas.html).

Statistical Analysis: One-way Nova test was used to estimate the association of genetic variations of the LPA gene with statin resistance in cardiovascular patients, as well as odds ratios (ORs) and 95 percent confidence intervals (CIs). A statistically significant p-value of 0.05 was used. SPSS was used for all statistical analysis.

RESULTS

To verify the allele specific extension strategy, the Sanger sequencing method was used to sequence the selected PCR products for two possible allelic variants of each genetic marker. The sequencing chromatograms for the allelic variants of 3 markers including rs**74617384,** rs**3798220 and** rs**3798872** of LPA gene. The results of sequencing chromatograms showed Figure 4.2A; Variant **G** of rs2306283, 4.2B; Variant **A** of rs2306283, 4.2C; Variant **T** of rs4149056, 4.2D; Variant **C** of rs4149056 shown 100% concordance with gel electrophoresis method in the selected samples.

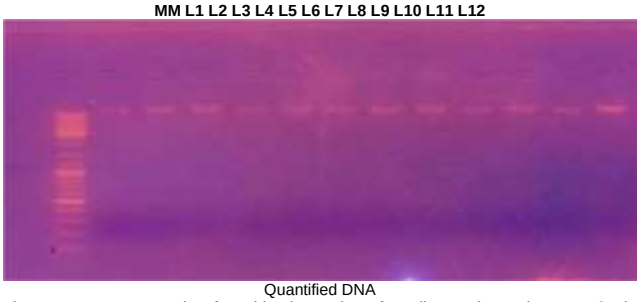


Figure 4.1: DNA extraction from blood samples of cardiovascular patients on Statin treatment (using 0.8% agarose gel)

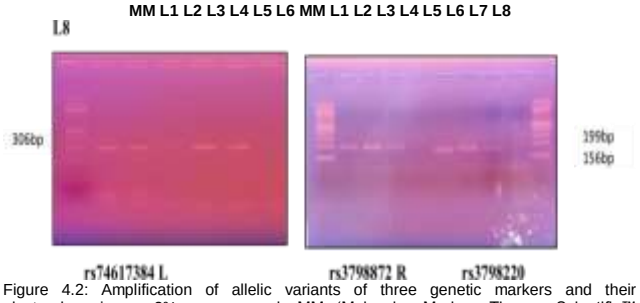


Figure 4.2: Amplification of allelic variants of three genetic markers and their electrophoresis on 2% agarose gel. MM (Molecular Marker; Thermo Scientific™ O'GeneRuler™ 100bp Plus DNA Ladder); Left L1, Variant A of rs74617384 (306bp); L2, Variant G of rs74617384 (306bp); L3, Variant A of rs74617384(359bp) Right; L1, Variant G of rs 3798872(156bp); L2, Variant A of rs3798872 (156bp); L3, Variant G of rs 3798872(156bp); L5Variant T of rs3798220 (199bp); L6, Variant C of rs3798220 (199bp); L7, Variant T of rs3798220 (199bp)

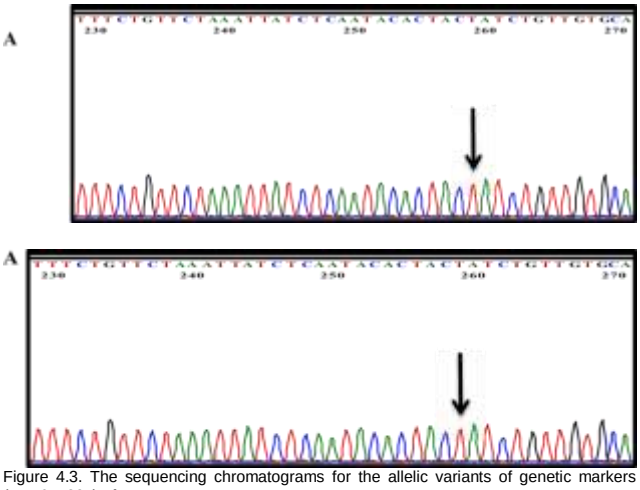


Figure 4.3. The sequencing chromatograms for the allelic variants of genetic markers (rs74617384) of LPA gene
A: LPA gene (Variant A of rs74617384)
B: LPA gene (Variant G of rs74617384)

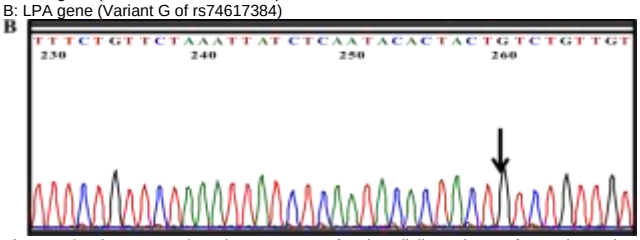
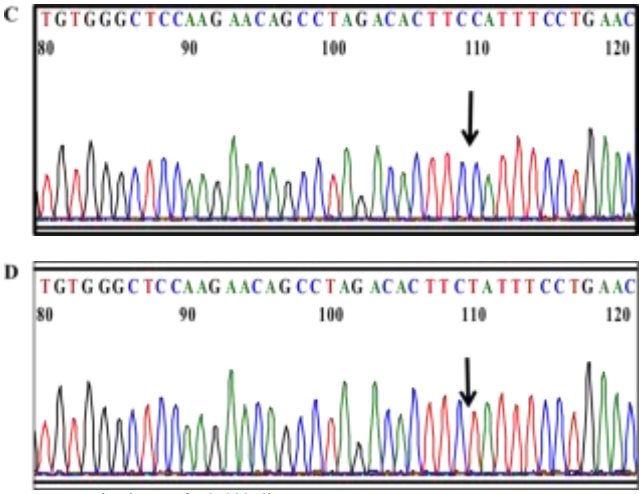


Figure 4.3. The sequencing chromatograms for the allelic variants of genetic marker (rs3798220) of LPA gene
C: LPA gene (Variant C of rs3798220)
D: LPA gene (Variant T of rs3798220)



E: LPA gene (Variant A of rs3798872)
F: LPA gene (Variant G of rs3798872)
Figure 4.3. The sequencing chromatograms for the allelic variants of genetic marker (rs3798872) of LPA gene

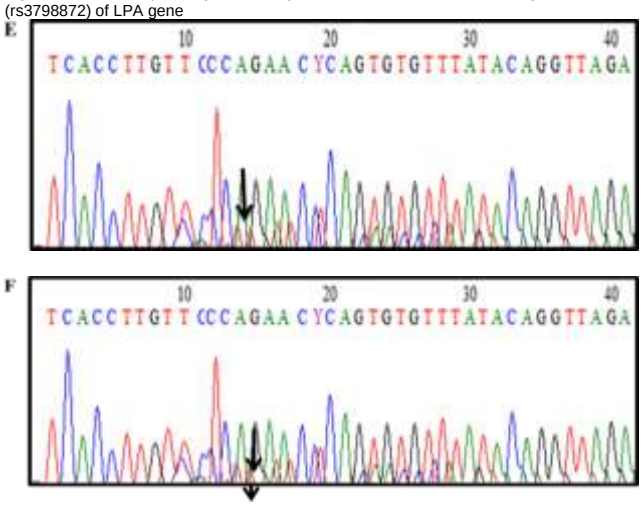


Table 4.1: Baseline characteristics of cardiovascular patients (n=90) on statin therapy (Mean values & Reference ranges)

Parameters	Mean ± Standard Deviation	Reference Ranges
Age (year)	50±12	
Total bilirubin (mg/dL)	0.66±0.24	0.2 – 1.2
Alanine aminotransferase (U/L)	38±18	0.0 – 40
Aspartate aminotransferase (U/L)	32±14	0.0 – 37
Alkaline phosphatase (U/L)	125±42	40 – 150
Albumin (g/dL)	4.8±0.2	3.5 – 5.0
Total Protein (g/dL)	7.2±0.2	6.4 – 8.3
Total Cholesterol (mg/dL)	164±37	< 200
Triglyceride (mg/dL)	167±72	< 150
High density Lipoprotein (mg/dL)	39±3.0	40 -60
Low density Lipoprotein (mg/dL)	95±37	<100
Very Low density Lipoprotein (mg/dL)	33±14	6 - 40
Creatine phosphokinase (U/L)	107±117	0.0 - 190
Lactate Dehydrogenase (U/L)	289±134	140 -480
Blood Urea (mg/dL)	35±18	10 - 50
Creatinine (mg/dL)	1.04±0.31	0.73 - 1.18 (Male) 0.55 – 1.02 (Female)

Table 4.2: The genotype fraction of genetic variant (SNP; rs10455872 of LPA gene) in cardiac patients of the study group

SNP;rs10455872 Genotypes	Number of Patients (n=90)	Genotype Fraction (%)
GA	60	0.66 (66.6)%
AA	18	0.2(0.20)%
GG	12	0.13(13.3)%

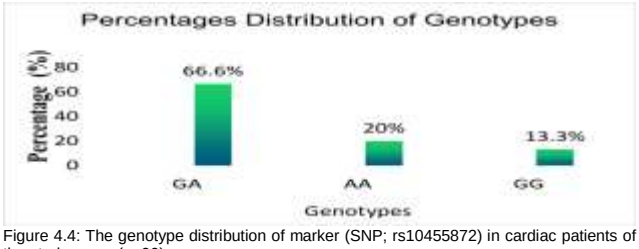


Figure 4.4: The genotype distribution of marker (SNP; rs10455872) in cardiac patients of the study group (n=90)

Table 4.3: The genotype fraction of genetic variant (SNP; rs3798220 of LPA gene) in cardiac patients of the study group.

SNP; rs3798220 Genotypes	Number of Patients (n=90)	Genotype Fraction (%)
TC	63	(0.7)70%
TT	12	(0.13)13.3%
CC	15	(0.16)16.6%

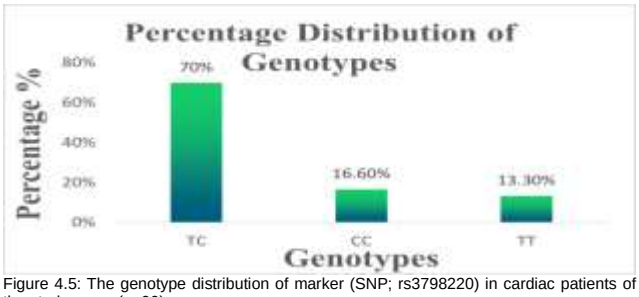


Figure 4.5: The genotype distribution of marker (SNP; rs3798220) in cardiac patients of the study group (n=90)

Table 4.4: The genotype fraction of genetic variant (SNP; rs74617384 of LPA gene) in cardiac patients of the study group

SNP; rs74617384 Genotypes	Number of Patients (n=90)	Genotype Fraction (%)
AG	75	(0.83)83.3%
GG	9	(0.10)10%
GT	3	(0.33)3.3%
AA	3	(0.33)3.3%

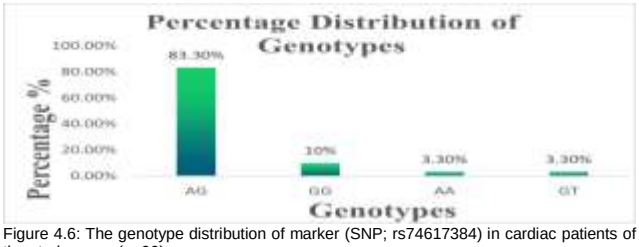


Figure 4.6: The genotype distribution of marker (SNP; rs74617384) in cardiac patients of the study group (n=90)

Table 4.5: The genotype distribution of genetic variant (SNP; rs10455872) in cardiac patients on statin treatment

SNP	Genotypic distribution of cardiac patients (n=90)			
SNP; rs10455872 G>A	Statin Salt (%)	GA	AA	GG
	Atorvastatin	(0.3)30%	0.1313.3%	(0.33)33%
	Rosuvastatin	0.36 36.6%	0.066.66%	0.1110%

*Most commonly statin salts prescribed by Physician.

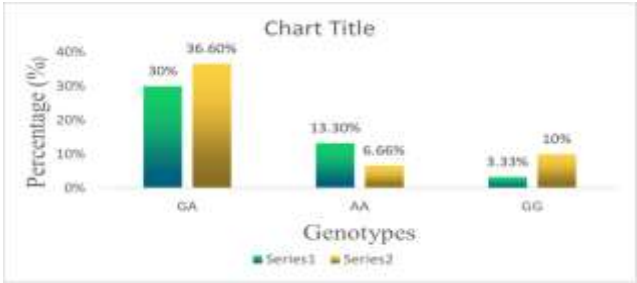


Figure 4.7: Graph showing percent genotype distribution of genetic variant (SNP; rs10455872) in cardiac patients on statin treatment i.e. Atorvastatin & Rosuvastatin

Table 4.6: The genotype distribution of genetic variant (SNP; rs3798220) in cardiac patients on statin treatment

SNP	Genotypic Distribution of Cardiac patients (n=90)			
SNP; rs 3798220 T>C	Statin Salt (%)	TC	TT	CC
	Atorvastatin	(0.26) 26.6%	(0.1) 10%	(0.1) 10%
	Rosuvastatin	(0.43) 43.3%	(0.03) 3.33%	(0.06) 6.66%

*Most commonly statin salts prescribed by Physician.

Table 4.7: The genotype distribution of genetic variant (SNP; rs74617384) in cardiac patients on statin treatment

SNP	Genotypic Distribution of Cardiac patients (n=90)			
SNP;rs 74617384 A>G	Statin Salt (%)	AG	GG	AA
	Atorvastatin	(0.36) 36.6%	(0.02) 2.22%	(0.01) 1.11%
	Rosuvastatin	(0.46) 46.6%	(0.01) 1.11%	0

*Most commonly statin salts prescribed by Physician.

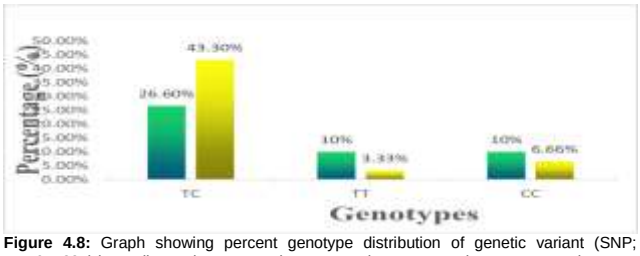


Figure 4.8: Graph showing percent genotype distribution of genetic variant (SNP; rs74617384) in cardiac patients on statin treatment i.e. Atorvastatin & Rosuvastatin

Table 4.8: Genotype wise clinic pathological data of genetic variant (Gene LPA; SNP rs10455872) of cardiac patients on statin treatment (n=90).

Gene LPA SNP rs10455872 (G>A)	Genotype GA	Genotype AA	Genotype GG
Number of patients	60	18	12
Total bilirubin (mg/dL)	0.69 ± 0.24	0.6± 0.21	0.725 ± 0.35
Alanine aminotransferase (U/L)	38.45 ± 20.68	41.33 ± 14.59	32.25 ± 14.66
Aspartate aminotransferase (U/L)	32.7 ± 15.45	31.66 ± 12.01	29.25 ± 13.67
Alkaline phosphatase (U/L)	103.9 ± 39.60	107.66± 40.03	115.5 ± 61.14
Albumin (g/dL)	4.16 ± 0.226	4.0 ± 0.17	4.3 ±0.21
Total Protein (g/dL)	7.26 ± 0.24	7.16 ± 0.23	7.3± 0.141
Total Cholesterol (mg/dL)	166.65 ± 37.33	158.33 ± 44.45	167.75 ± 35.45
Triglyceride (mg/dL)	156.2 ±76.74	156.83 ± 70.01	214.75± 47.59
High density Lipoprotein (mg/dL)	46.95 ± 3.68	42 ± 3.22	37.75 ± 3.30
Low density Lipoprotein (mg/dL)	100.45 ± 39.77	88.16 ± 28.20	95.25 ± 49.11
Very Low Density Lipoprotein(mg/dL)	31.55 ± 14.79	31.16 ± 14.05	43 ± 9.34
Creatine phosphokinase (U/L)	118.9 ± 131.02	49.5± 21.09	152.75 ±126.72
Lactate Dehydrogenate (U/L)	307.25± 148.39	228.66 ± 110.60	351.75± 57.86
Blood Urea (mg/dL)	37.8 ± 18.84	40.83 ± 18.50	35.75 ± 13.64
Creatinine (mg/dL)	1.04 ± 0.24	1.18± 0.47	1.025 ± 0.22

Table 4.9: Genotype wise clinic pathological data of genetic variant (Gene LPA; SNP rs3798220) of cardiac patients on statin treatment (n=90).

Gene LPA SNP rs3798220 (T>C)	Genotype TC	Genotype CC	Genotype TT
Number of patients	63	5	4
Total bilirubin (mg/dL)	0.72 ± 0.26	0.66 ± 0.15	0.47 ± 0.23
Alanine aminotransferase (U/L)	35.38 ±18.43	50.2 ± 18.45	38± 14.44
Aspartate aminotransferase (U/L)	29.80 ±12.93	39.6 ± 18.9	34.25 ± 12.5
Alkaline phosphatase(U/L)	132.57 ± 41.85	120 ± 17.7	85.5±50.79
Albumin (g/dL)	4.13± 0.22	4.1 ± 0.244	4.27 ± 0.22
Total Protein (g/dL)	7.29 ± 0.20	7.1 ± 0.35	7.2 ± 0.11
Total Cholesterol (mg/dL)	164± 27.16	183.2 ± 62.05	148.5 ± 45.36
Triglyceride (mg/dL)	153.19 ± 67.48	194.6± 86.62	183.5 ± 92.20
High density Lipoprotein (mg/dL)	40.14± 3.86	38.6 ± 1.81	41 ± 4.69
Low density Lipoprotein (mg/dL)	99.42 ±37.08	105.8± 46.78	75.5 ± 35.5
Very Low Density Lipoprotein (mg/dL)	30.85 ± 13.36	39.2 ± 17.1	36.5 ±18.55
Creatine phosphokinase (U/L)	124.4 ± 127.50	45.8 ± 18.22	111± 117.12
Lactate Dehydrogenate (U/L)	323.52±141.19	191.4 ± 114.7	293.25 ± 112.73
Blood Urea (mg/dL)	39.09 ± 24.24	32.6 ± 8.1	40 ± 25.17
Creatinine (mg/dL)	1.06± 0.27	1 ± 0.19	1.2 ± 0.63

This table presents us the clinical pathological association of different parameters with the genetic variants (rs10455872) of the Lpa gene.

This table presents us the clinical pathological association of different parameters with the genetic variants (rs**3798220**) of the Lpa gene.

Table 4.10.: Genotype wise clinic pathological data of genetic variant (Gene LPA SNP rs74617384) of cardiac patients on statin treatment (n=90)

Gene LPA SNP rs74617384(T>C)	Genotype GA	Genotype GG
Number of patients		
Total bilirubin(mg/dL)	0.65 ±0.24	0.96 ± 0.11
Alanine aminotransferase (U/L)	35.4± 15.20	53.33± 35.47
Aspartate aminotransferase (U/L)	31.08± 13.99	38.33 ± 20.00
Alkaline phosphatase(U/L)	123.08± 40.12	147.33 ± 56.19
Albumin(g/dL)	4.16± 0.20	3.96± 0.11
Total Protein(g/dL)	7.28± 0.21	7.13 ± 0.37
Total Cholesterol(mg/dL)	168.44 ± 38.37	159.33± 21.12
Triglyceride(mg/dL)	159.12 ± 76.43	171 ± 39.12
High density Lipoprotein(mg/dL)	40.4± 3.66	38 .33 ± 4.50
Low density Lipoprotein(mg/dL)	110.84 ± 37 .99	98 ± 33.77
Very Low Density Lipoprotein(mg/dL)	32± 15.15	34 .33± 8.14
Creatine phosphokinase(U/L)	98.63± 115.21	168.66 ± 109.86
Lactate Dehydrogenate(U/L)	292.2± 137.51	392.33 ± 149.63
Blood Urea (mg/dL)	38.4 ± 23.28	46.33 ± 14.15
Creatinine(mg/dL)	1 ± 0.33	1.1 ± 0.26

This table presents us the clinical pathological association of different parameters with the genetic variants (rs74617384) of the Lpa gene.

The genotyping of Three genetic variants of Gene LPA (SNPs; rs74617384, rs3798220 and 10455872) were performed for a total of 90 cardiac patients who were on statin therapy from at least last one year. For SNP (rs10455872), the genotype frequencies of genetic variant GA (66.6%), AA (20%), and GG (13.3%) were observed. The genotype GA (66.6%) was found to be the most frequent genotype and GA (13%) the least frequent genotype in this study group.

The genotype based stratified data for clinic pathological parameters in SNP; (rs10455872), total triglycerides observed in the genotype GG were higher (mean value 214 mg/dL, above the normal range <150 mg/dL) than the genotype GA (mean value 156 mg/dL) and genotype AA mean value 156.83.5mg/dL of cardiac patients of this study. However, the Low Density Lipoprotein (LDL), and Lactate Dehydrogenase (LDH), more important criteria regarding the statin therapy response LDL were higher in genotype GA (mean value 100.45 mg/dL in comparison to genotype AG and AA (mean value 95 mg/dL & 88.16 mg/dL respectively). Lactate dehydrogenase was higher in genotype GG mean value 351.75 mg/dL in comparison to genotype GA and AA (307.25 &228.66). To evaluate renal insufficiency in response to statin resistance in cardiac patients, important parameters, Creatine phosphokinase (CPK), Serum Blood Urea levels and Blood Urea Nitrogen (BUN) were determined for genotype of SNP (rs10455872) in the study group. CPK was higher in GG genotype (mean value 152.75 U/L in comparison to GA genotype and AA (mean value 118.9U/L and 49 U/L respectively). Blood urea nitrogen (BUN) was higher in AA genotype (mean value 40.83 U/L in comparison to GA genotype and GG (mean value 37.8U/L and 35.75U/L respectively for SNP (rs10455872).

In SNP (rs3798220), the genotype frequencies of genetic variant TC 70%), CC 16.3%, and TT 13.3 % were observed. The TC 70% was found to be the most frequent genotype and CC (16.3%) & TT (13.3%) the least frequent genotypes in this study group.

The genotype based stratified data for clinic pathological parameters in SNP; (rs3798220), total triglycerides observed in the genotype CC were higher (mean value 194.6 mg/dL, above the normal range <150 mg/dL) than the genotype TT (mean value183.5 mg/dL) and genotype TC mean value 153.19 mg/dL of cardiac patients of this study. However, the Low Density Lipoproteins (LDL), and Lactate Dehydrogenase (LDH), more important criteria regarding the statin therapy response. The Low

Density Lipoproteins (LDL) were higher in genotype TT (mean value 105.8 mg/dL) in comparison to genotype TC and genotype CC (mean value 99.42 mg/dL & 75.5 mg/dL respectively). Lactate dehydrogenase was higher in genotype TC (323.52mg/dL) in comparison to other genotypes TT and CC (293.25mg/dL &191.4 mg/dL) To evaluate renal insufficiency in response to statin resistance in cardiac patients, important parameters, Creatine phosphokinase (CPK), Serum Blood Urea levels and **Blood Urea Nitrogen (BUN)** were determined for genotypes of SNP (rs3798220) in the study group. CPK was higher in TC genotype (mean value 124.4 U/L in comparison to other 2 genotypes CC genotype and TT (mean value 45.5 &111 respectively) for SNP (rs3798220).

In SNP (rs74617384), the genotype frequencies of genetic variant AG (83.2%), GG (10%,) GT (3.3%) were observed. The AG (83.2) 7 was found to be the most frequent genotype and GG (10%) & GT (3.3%) the least frequent genotype in this study group.

The genotype based stratified data for clinic pathological parameters in SNP; (rs74617384), total triglycerides observed in the genotype GG (mean value 171mg/dL, above the normal range <150 mg/dL) and AG (mean value 159mg/dL, above the normal range <150 mg/dL) found in the cardiac patients of this study. The serum LDL levels and LDH levels are also considered important criteria regarding the statin therapy response. The serum LDL levels were found to be higher than normal values in genotype GA (mean value 110 mg/dL; normal values <100 mg/dL) in comparison to other genotypes of SNP (rs74617384) found in the study group. However, lactate dehydrogenase levels were higher in genotype GG (mean value 392.33mg/dL) in genotype AC of SNP (rs74617384) in the studied group of cardiac patients.

To evaluate renal insufficiency in response to statin resistance in cardiac patients for the genetic marker, rs74617384, Creatine phosphokinase (CPK), Serum Blood Urea levels and Blood Urea Nitrogen (BUN) were also determined. Both CPK and BUN was higher in GG genotype (mean value 168.66 U/L & 46.33 respectively) in comparison to all other genotype of marker, rs74617384found in this study.

Table 4.11: Association of genetic variants of LPA gene *(rs10455872 rs3798220 rs74617384*) with statin resistance (i.e. Physician's prescribed salt) using critical biochemical parameters.

Statin Salt	*rs10455872*						*rs3798220*						
	GENOTYPE	GA	AA	*p-value*	GG	*p-value*	TC	TT	P-value	*CC*	P-VALUE	AG	*GG*
Atorvastatin	No. of patients	27	9	-----	6	-----	33	3		6		33	9
	Lactate Dehydrogenasel Mean	406.77	153.66	P<0.0001	369	0.5	426.375	126	_	246.5	0.01	526	
	Triglyceride Mean	183.72	152.66	0.1	213	0.6	173.90	96	_	262.5		196.25	
	Creatine phosphokinase mean	125.81	40.33	0.05	132	0.6	160.45	72	_	39.5	0.09	167.37	
	Low density Lipoprotein *mean*	113.54	89.66	0.2	110.5	0.8	106.54	46	_	149.5	0.03	113.62	
	BLOOD UREA NITROGEN Mean	50.33	48	0.8	44.5	0.6	48.45	44	_	39.5	0.4	49.72	0
Rosuvastatin	No. of patients	33	9	-----	6	-----	30	9		9		42	0
	Lactate Dehydrogenasel Mean	225.8	303.5	0.06	334.5	0.02	270.33	349	0.06	154.66	0.001		
	Triglyceride Mean	139.78	161	0.03	216.5	0.006	138.37	212.66		149.33	0.004	134	0.6
	Creatine phosphokinase mean	77.35	58.66	0.1	173.5	0.004	93.62	132.66	0.14	50	0.01	79.36	
	Low Density Lipoprotein *mean*	80.87	86.66	0.9	80	0.6	91	76.66	0.2	76.66	0.2	86.90	
	BLOOD UREA NITROGEN Mean	27.54	33.66	0.04	27	0.8	28.5	21	0.001	28	0.8	29.5	46.33

The genotype based stratified data for clinic pathological parameters in SNP; (rs74617384), total triglycerides observed in the genotype GG (mean value 171mg/dL, above the normal range <150 mg/dL) and AG (mean value 159mg/dL, above the normal range <150 mg/dL) found in the cardiac patients of this study. The serum LDL levels and LDH levels are also considered important criteria regarding the statin therapy response. The serum LDL levels were found to be higher than normal values in genotype GA (mean value 110 mg/dL; normal values <100 mg/dL) in comparison to other genotypes of SNP (rs74617384) found in the study group. However, lactate dehydrogenase levels were higher in genotype GG (mean value 392.33mg/dL) in genotype AC of SNP (rs74617384) in the studied group of cardiac patients.

To evaluate renal insufficiency in response to statin resistance in cardiac patients for the genetic marker, rs74617384, Creatine phosphokinase (CPK), Serum Blood Urea levels and Blood Urea Nitrogen (BUN) were also determined. Both CPK and BUN was higher in GG genotype (mean value 168.66 U/L & 46.33 respectively) in comparison to all other genotype of marker, rs74617384found in this study.

DISCUSSION

Cardiovascular patients, who did not attain critical biochemical parameters are considered to be statin-resistant (Stroes *et al*., 2015). Differences in drug absorption, transport, intrahepatic drug metabolism, drug metabolism in other organs, and drug excretion mechanisms can all be attributed to statin resistance. Some environmental factors can influence the biochemical parameters response to statins. Patients with hypertension and smoking habits have a smaller decrease in the biochemical parameters than those without hypertension and nonsmoking habits (Greenland *et al.,* 2001). Numerous single nucleotide polymorphism (SNP's) have been found in solute carrier organic transporter LPA gene, three common SNP's labeled as 872 G>A, 220T>C 384 A>G and which are considered the most frequent and responsible for suitable transport function of LPA transporter. These three LPA single nucleotide polymorphisms (SNPs), 872 G>A, 220T>C 384 A>G are found commonly in many ethnic groups, and their association with clinical pharmacokinetics had been examined by researchers in different areas of world.

The Pakistani cardiac patients (both Males and Females i.e. n=90) who were on Statin therapy from last one year became the subjects of this study to evaluate the statin therapeutic response. The allele specific PCR extension was performed to amplify and genotype the targeted region of SNPs of gene LPA. The gel based identified genotypes in the SNPs were further validated by Sanger sequencing. For SNP (rs104455872), the genotype GA (66.6%) was found to be the most frequent genotype in comparison to genotype AA (20%) and GG (13.3%) in this study group. Similarly, for genetic marker (SNP rs3798220) the genotype frequencies of genetic variant TC (70%), is higher as compared to other genotypes TT (13.30%), CC (16.6%). For genetic marker rs 74617384) AG was found higher in the genotype AG (83.3%) in comparison to other genotype GG (10%) to other minor allele. In overall cardiac patients, the Rosuvastatin was prescribed to more cardiac patients (55.17%) than the atorvastatin (44. 83%).The genotype GA (rs104455872) was more frequently prescribed Rosuvastatin by the physician (36.3%) in comparison to atorvastatin (30%) for the same genotype GA. All other two

genotypes of marker rs104455872 were least frequently prescribed with both statin salts (Rosuvastatin & atorvastatin). Similarly, the genotype TC of marker, (rs3798220), was more frequently prescribed rosuvastatin by the physician (43.3%) in comparison to atorvastatin (26.6%) for the same genotype TC. The genotype TT of marker, rs3798220, found rarely prescribed by both statin salts i.e. rosuvastatin (0.0%) & atorvastatin (3.4%).The genotype AG (rs74617384) was more frequently prescribed rosuvastatin by the physician (46.6%) in comparison to atorvastatin (36.6%) for the same genotype AG. All other three genotypes of marker rs2306283 were least frequently prescribed with both statin salts (rosuvastatin & atorvastatin).

The association of genetic variants of LPA gene with (rs104455872), (rs3798220) or (rs 74617384) statin resistance was evaluated by considering the levels of five critical biochemical parameters (Triglycerides, Low Density Lipoproteins, Creatine phosphokinase, Lactate Dehydrogenase & Blood Urea Nitrogen) with respect to statin therapeutic response in cardiac patients. The levels of five clinical biochemical parameters have been recommended in previous literature as critical parameters to evaluate the therapeutic response of statin in cardiac patients (Gary *et al.*, 2015).

In statin salt and genotype based stratified data of cardiac patients of this study, the patients having all genotypes of marker, rs104455872, treated by an atorvastatin showed higher (mean values: GG; mg/dL 214.75, AA;156.88mg/dL, GA; 156.2 mg/dL, than normal values of triglycerides (<150mg/dL). Triglycerides (TGs) most common type of fat in the body, are not directly atherogenic but represent an important biomarker of CVD risk because of their association with proatherogenic plasma proteins LDL and VLDL (Talayero *et al.,* 2011). The renal insufficiency is also another important criterion to observe the statin treatment response in cardiac patients. The serums levels of Creatine PhosphoKinase (CPK), blood urea and Blood Urea Nitrogen (BUN) are recommended parameters in cardiac patients to evaluate the renal functions in general as well as in response to statin therapy The Blood urea and Serum blood urea nitrogen (BUN) have been reported as easily determinable and inexpensive markers to identify patients at high risk for vascular end points (Gary *et al*., 2015). Moreover, blood urea has been also considered as an independent prognostic factor in severity of aortic stenosis (AS) in heart failure patients (Haberman *et al.,* 2020). Statin-associated myopathy, defined as a significant increase in serum creatine kinase (CK), is a rare but serious side effect of statins that affects 1 in 1000 to 1 in 10,000 people taking standard statin doses (Stroes *et al.*, 2015). Creatine kinase (CK) has been recommended as a diagnostic marker for statin-associated muscle symptoms to determine which patients are truly statin intolerant and require alternative cholesterol-lowering medications (SAMS). An increase in CK with statin therapy may help distinguish between patients with true SAMS and those with non-specific muscle pain (Stroes *et al*., 2015).

The withdrawal of cerivastatin in 2001, after it caused approximately 100 deaths dueto rhabdomyolysis, demonstrates the importance of statin-induced muscle toxicity (skeletal muscle fiber breakdown with leakage of muscle contents into the circulation) (Jamal et al ., 2004). Substantial CPK increases and rhabdomyolysis have been reported with statin use, particularly in patients starting treatment, those on high daily doses or interacting drugs (reflecting higher systemic exposure), patients on multiple concomitant drugs, and those taking rosuvastatin. (Van Staa *et al*., 2014).

Concerns have been raised about statin safety in terms of myopathy and rhabdomyolysis, which have been linked to increased activity of both CPK and Lactate Dehydrogenase (Habte *et al*., 2020). The evaluation of serum LDH has been considered an important diagnostic and prognostic marker to monitor the cardiac disease such as myocardial infarction (Habte *et al*., 2020). Lactate dehydrogenase (LDH) is a cytoplasmic enzyme found in almost all major organ systems, particularly the heart and liver muscles. LDH release to extracellular space is primarily caused by the ingestion of certain drugs, toxins, and chemical poisons. (Elias *et al*., 2018). As a result, elevated serum LDH may serve as a biomarker for the diagnosis of drug induced hepatic and muscular injury.

Statin therapy has been shown in previous studies to be responsible for moderate-to-severe elevations in serum LDH (Noor *et al.,* 2016). Triglyceride show more resistance than the four biochemical parameters in all the three genetic markers when treated with atorvastatin and rosuvastatin. LDL levels were higher than the normal range in all three genetic markers when treated with atorvastatin as compared to rosuvastatin. CPK levels were also higher than the normal range in all variants when treated with the atorvastatin as compared to rosuvastatin.

As per previous literature, the remaining four critical biochemical parameters including LDL, CPK, LDH & BUN, showed adverse atorvastatin therapeutic response (mean values are higher than normal values) in genotypes of marker, rs10455872 (GA& GG), in comparison to genotypes (rs10455872; AA in cardiac patients of this study. In rosuvastatin treated patients, two parameters LDH and TG of two genotypes (rs10455872; AA& GG) were seen above the normal range.

For genetic marker rs3798220, the genotype based stratified data of cardiac patients showed that the atorvastatin therapeutic response for the patients having genotype TCof this marker was adverse with respect to four critical parameters (LDL, CPK, LDH & BUN) in comparison to TT and CC genotype. It has been suggested in previous studies that LDL is more proatherogenic than VLDL (Van Craeyveld *et al.,* 2010), supporting our data regarding high values of LDL and low values of VLDL in the patients having genotype TT for adverse therapeutic effects of atorvastatin.

Moreover, in rosuvastatin treated patients, single genotype (rs3798220; TT) show adverse effects in comparison to other two genotype (TC and CC) for atorvastatin treated patients. BUN values were found above in all the three genotypes when treated with atorvastatin For genetic marker rs (74617384) only one genotype AG values were above the normal range when treated with atorvastatin in comparison to other two genotypes GG and AA. Whereas, in rosuvastatin only one genotype (AG) was found and the values were in normal range.

CONCLUSION

The frequency distribution of genetic markers of gene LPA (rs104455872), (rs3798220) or (rs 74617384) showed that the genotype GA 66.6%, TC (70%) and AG (83.3%) respectively, were found to be the most frequent genotypes in our cardiac patients. To evaluate the statin resistance in the cardiac patients by genotype based stratified data, levels of five critical biochemical parameters were considered (Triglycerides, Low Density Lipoproteins, Creatine phosphokinase, Lactate Dehydrogenase & Blood Urea Nitrogen). These critical biochemical parameters evidenced the adverse effects of atorvastatin and rosuvastatin (statin salt resistance) in patients having genotypes GA of marker, rs10455872, in comparison to genotypes AA & GG. In rosuvastatin treated patients, genotype GG and AA (rs10455872 shows adverse effects in comparison to other GA genotype.

For marker rs3798220, TC genotype showed adverse effects (statin salt resistance) with respect to three critical parameters (LDL, CPK, LDH) in comparison to CC and TT genotype in atorvastatin treated cardiac patients. Moreover, in rosuvastatin treated patients, genotype (TT) of marker rs3798220 show adverse effects in comparison to other two genotypes (TC and CC).

For marker (rs74617384) in both the atorvastatin and rosuvastatin treated patients only one genotype (AG) were found whose values were above the normal range in comparison to other genotype (GG) when treated with atorvastatin. Whereas in rosuvastatin only one genotype (AG) were found whose values were in normal range Hence, the genotyping of cardiac patients for the genetic markers of gene LPA(rs104455872), (rs3798220) or (rs

74617384) may be helpful to tailor the statin salt for precision therapy and may reduce the adverse effects in cardiac patients

ABBREVATIONS

Snp : Single nucleotide polymorphism
CVD : Cardio vascular disease
LPA : lipo protein particle A
HMG CoA :Hydroxy methyl glutarate co enzyme A
SAMS : Statin Associated Muscle Symptoms
LDL: Low density lipoprotein
HDL : High density lipoprotein

REFRENCES

1. Akao, H., Polisecki, E., Kajinami, K., Trompet, S., Robertson, M., Ford, I., ... & Schaefer, E. J. (2012). KIF6, LPA, TAS2R50, and VAMP8 genetic variation, low density lipoprotein cholesterol lowering response to pravastatin, and heart disease risk reduction in the elderly. *Atherosclerosis*, ***220***(2), 456-462.
2. Backes, J. M., Ruisinger, J. F., Gibson, C. A., & Moriarty, P. M. (2017). Statin-associated muscle symptoms—managing the highly intolerant. *Journal of clinical lipidology*, ***11***(1), 24-33.
3. Cappuccio, F. P., Cooper, D., D'Elia, L., Strazzullo, P., & Miller, M. A. (2011). Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *European heart journal*, ***32***(12), 1484-1492.
4. Chasman, D. I., Shiffman, D., Zee, R. Y., Louie, J. Z., Luke, M. M., Rowland, C. M., ... & Ridker, P. M. (2009). Polymorphism in the apolipoprotein (a) gene, plasma lipoprotein (a), cardiovascular disease, and low-dose aspirin therapy. *Atherosclerosis*, ***203***(2), 371-376.
5. Greenland, P., Smith Jr, S. C., & Grundy, S. M. (2001). Improving coronary heart disease risk assessment in asymptomatic people: role of traditional risk factors and noninvasive cardiovascular tests. *Circulation*, *104*(15), 1863-1867
6. Habte, M. L., Melka, D. S., Degef, M., Menon, M. K. C., Yifter, H., & Feyisa, T. O. (2020). Comparison of lipid profile, liver enzymes, creatine kinase and lactate dehydrogenase among type ii diabetes mellitus patients on statin therapy. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *13*, 763.
7. Hermann, M., Bogsrud, M. P., Molden, E., Åsberg, A., Mohebi, B. U., Ose, L., & Retterstøl, K. (2006). Exposure of atorvastatin is unchanged but lactone and acid metabolites are increased several-fold in patients with atorvastatin-induced myopathy. *Clinical Pharmacology & Therapeutics*, ***79***(6), 532-539.
8. Hirotsu, N., Murakami, N., Kashiwagi, T., Ujiie, K., & Ishimaru, K. (2010). Protocol: a simple gel-free method for SNP genotyping using allele-specific primers in rice and other plant species. *Plant Methods*, *6*(1), 1-10.
9. Hopewell, J. C., Parish, S., Offer, A., Link, E., Clarke, R., Lathrop, M. & MRC/BHF Heart Protection Study Collaborative Group. (2013). Impact of common genetic variation on response to simvastatin therapy among 18 705 participants in the Heart Protection Study. *European heart journal*, ***34***(13), 982-992.
10. Li, Y., Luke, M. M., Shiffman, D., & Devlin, J. J. (2011). Genetic variants in the apolipoprotein (a) gene and coronary heart disease. *Circulation: Cardiovascular Genetics*, ***4***(5), 565-573.
11. Li, Z., Zhang, Z., He, Z., Tang, W., Li, T., Zeng, Z., ... & Shi, Y. (2009). A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis. bio-x. cn). *Cell research*, *19*(4), 519-523.
12. Maniatis, T., & Fritsch, E. F. (1982). Sambrook. *M0lecular Cloning-A Laboratory Manual, Cold Water Spring Harbour p464*.
13. Menotti, A., Alberti-Fidanza, A., Fidanza, F., Lanti, M., & Fruttini, D. (2012). Factor analysis in the identification of dietary patterns and their predictive role in morbid and fatal events. *Public health nutrition*, ***15***(7), 1232-1239.
14. Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., ... & Turner, M. B. (2016).Executive summary: heart disease and stroke statistics—2016 update: a report from the American Heart Association. *Circulation*, ***133***(4), 447-454.
15. Noor, M., Waheed, A., Muhammad, I., & Bakhtiar, S. (2016). Is co q10 really valuable in shielding statin induced myopathy-an exploration. *PAFMJ*, ***66***(4), 525-29.
16. Schachter, M. (2005). Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundamental & clinical pharmacology*, ***19***(1), 117-125.
17. Song, H., Fang, F., Arnberg, F. K., Mataix-Cols, D., de la Cruz, L. F., Almqvist, C., ... & Valdimarsdóttir, U. A. (2019). Stress related disorders and risk of cardiovascular disease: population based, sibling controlled cohort study. *Bmj*, *365*.
18. Stroes, E. S., Thompson, P. D., Corsini, A., Vladutiu, G. D., Raal, F. J., Ray, K. K., ... & Wiklund, O. (2015). Statin-associated muscle symptoms: impact on statin therapy—European Atherosclerosis Society consensus panel statement on assessment, aetiology and management. *European heart journal*, *36*(17), 1012-1022.
19. Talayero, B. G., & Sacks, F. M. (2011). The role of triglycerides in atherosclerosis. *Current cardiology reports*, *13*(6), 544-552.
20. Van Staa, T. P., Carr, D. F., O'Meara, H., McCann, G., & Pirmohamed, M. (2014). Predictors and outcomes of increases in creatine phosphokinase concentrations or rhabdomyolysis risk during statin treatment. *British journal of clinical pharmacology*, *78*(3), 649-659.