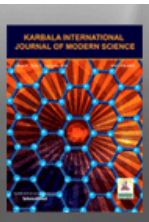

Karbala International Journal of Modern Science

Volume 6 | Issue 1 Article 7

A Study to Explore the LDLR Gene Polymorphisms Contribute to Atorvastatin Response in a Sample of Iraqi Population with Atherosclerotic Coronary Artery Disease

Shaimaa Y. Abdulfattah
Al-Nahrain University, Baghdad, Iraq, shaimaay26@gmail.com

Salwa J. Abdullah
Al-Nahrain University, Baghdad, Iraq, fsalwaj@yahoo.com

Hilal B. Alsaffar
University of Baghdad, Baghdad, Iraq, hilal.alsaffar@meciq.edu.iq

Recommended Citation

Abdulfattah, Shaimaa Y.; Abdullah, Salwa J.; and Alsaffar, Hilal B. (2020) "A Study to Explore the LDLR Gene Polymorphisms Contribute to Atorvastatin Response in a Sample of Iraqi Population with Atherosclerotic Coronary Artery Disease," *Karbala International Journal of Modern Science*: Vol. 6 : Iss. 1 , Article 7.
Available at: https://doi.org/10.33640/2405-609X.1361

A Study to Explore the LDLR Gene Polymorphisms Contribute to Atorvastatin Response in a Sample of Iraqi Population with Atherosclerotic Coronary Artery Disease

Abstract

Genetic factors will determine the higher variability, which found in response to lipid reduction treatment (statins). However, due to ethnicity the frequency and effect of single nucleotide polymorphisms (SNPs) may differ. The main aim of lipid lowering medical treatment is to eventually prevent the endogenous production of cholesterol through inhibition of HMG-CoA reductase, The resulting decrease in hepatocyte cholesterol concentration triggers up-regulation of low-density lipoprotein (LDL)-receptor expression by inducing sterol regulatory element-binding protein (SREBP) 2 cleavage, as SREBP-2 activates LDL receptor transcription. The aim of this study was to evaluate the effects of low density lipoprotein receptor (*LDL-R*) gene variants (rs200727689; rs72658860) in response to atorvastatin treatment in atherosclerotic coronary artery disease (ACAD) in a sample of Iraqi patients. The genetic polymorphisms of rs200727689 and rs72658860 were studied in patients undergoing coronary angiography (CAG). One hundred Iraqi patients include 52 patients treated with 20mg/day and 48 patients treated with 40mg/day, In addition, 100 apparently healthy subjects were genotype for these SNP by the thermal profile of allelic discrimination methods used Real Time PCR (RT-PCR) assay. A significant increase in *A* allele frequency (rs200727689) compared with controls for total ACAD patients (43% vs 23.5%; OR= 2.46; 95% C.I: 1.60-3.77; p=0.000). For (rs72658860) SNP, among total patients the *A* allele also increased the frequency substantially compared with the controls (40.5% vs 23.5%; P=0.0003; OR= 2.22; 95% C.I: 1.44 -3.41). In the patients who were treated with atorvastatin, SNP rs72658860 AA genotype significantly affected the response of atorvastatin in dose 20mg compared with 40mg. The result showed in AA genotype of (rs200727689) SNP a reduction in TC concentrations (277.55±108.38 vs 326.99±63.56) and LDL-C concentrations (198.33±100.0 vs 250.01±62.52) in sera compared with GG genotype in patients treated with 20 mg atorvastatin, although, none in all parameters statistically significant differences.

Keywords

Keywords: Atherosclerosis, Atrovastatin, Genotype, Low density lipoprotein receptor, Pharmacogenetic, Single nucleotide polymorphism.

Cover Page Footnote

We kindly thank all the individuals for their cooperation and participation in the study and the cooperation of the medical staff at Surgical specialist (Ghazi Al -Hariri) Hospital/ Baghdad, Iraq

1. Introduction

The elevated plasma concentration of low-density lipoprotein cholesterol (LDL-C) was highly correlated as a major risk factor of coronary heart disease (CHD). Clinical management of hypercholesterole with statins not only improves the lipid profile, but also has numerous atheroprotective impacts, decreasing cardiovascular mortality and morbidity [1]. Statins are used for the treatment of cardiovascular disease and are one of the most prescribed medication groups. Their influence through HMGCR inhibition results decreased intrahepatic cholesterol synthesis, hepatocyte surface upregulation of low-density lipoprotein cholesterol (LDL-C) receptors, increased hepatocyte LDL-C uptake, and decreased systemic LDL-C concentration [2].

Atorvastatin is used as a statin therapy abroad with better safety and tolerance to lower the serum lipid level, but some patients responded very poorly to the statin therapy. Genome-wide association study (GWAS) was observed the (single nucleotide polymorphisms) SNPs is related not just to the blood lipid level, but also to the therapeutic effect of statin and a spectrum of various responses among treated individuals were emerging [3]. This inter-individual variation in therapeutic response can be determined by several factors, including physiological and biological conditions for patients, adherence to treatment, age, gender, ethnicity and specific genetic factors for individuals [4].

In this context, the Pharmacogenetics (the branch of pharmacology) discusses the role of genetic variation in the drug response of patients by correlating gene expression or single nucleotide polymorphisms (SNP) with the efficacy or toxicity of a drug. With regard to the patient's genotype, it is opposed to the growth of rational means to enhance drug therapy with minimal side effects to maintain high efficacy [5]. Atorvastatin is a class of statins used in Iraqi healthcare services, screening for the response of atorvastatin pharmacogenetics markers to the Iraqi property population is of higher interest, but some of the patients responded very poorly to the treatment of statin [6].

In additon, the two polymorphisms (rs200727689; rs72658860) assessed in this study as the low-density lipoprotein receptor (LDL-R) is substantially associated with variability in the serum LDL-C level and statin response.

2. Materials and methods

2.1. Participants

This study was conducted in two hundred male and female participants, their ages ranged between (30−60) years, included ACAD patients (n = 100) and classified into two subgroups; patients treated with 20 mg atorvastatin (n = 52), and patients treated with 40 mg (n = 48) of atorvastatin therapy and they were diagnosed according to the WHO criteria [7], based on clinical examination, including chest pain plus either electrocardiographic (ECG), echocardiography, treadmill test (TMT), and they have been asked to complete clinical history of myocardial infarction (MI), angina pectoris (stable and unstable). A structured questionnaire was used to identify cardiovascular risk factors such as lipid profile, blood pressure, body mass index (BMI) mass (Kg)/height (m)2, diabetes mellitus (FBS), age and gender. Patients were selected from the Iraqi center for heart disease at Ghazi Al-Hariri hospital/ Baghdad-Iraq for diagnosis and treatment. Neither of the participants had diabetes hepatic, kidney endocrinological or malignant disease, nor they were receiving lipid-lowering associated treatment. In addition, apparently healthy participants of control (n = 100) were randomly selected for routine examinations.

2.2. Biochemical determinations

After a fast 12-h overnight, venipuncture was used to get blood samples. Biochemical analysis was calculated using conventional enzyme-colorimetric (Biolabo/France) techniques and measured low-density lipoprotein cholesterol using Friedewald formula on which triglycerides did not exceed 400 mg/dL (4.8 mmol/L). Pathological, normal and commercial serums (Biolabo/France) have been used to verify the validity of biochemical results.

2.3. Genetic and molecular analysis

Wizard Genomic DNA Purification Kit (Promega, USA) was used to extract DNA from EDTA blood samples. Genotyping was conducted by Taqman allelic discrimination Real-time PCR assay. DNA isolation was subjected for RT-PCR amplification of two SNPs in *LDL-R* gene. The sequence of each primers and

https://doi.org/10.33640/2405-609X.1361

Table 1
Primers and Probes of *LDL-R* gene polymorphisms used in the study.

Primer/probes	Sequence (5 ′–3 ′)	Product size bp
LDL-R rs200727689	F:5 ′ -GCCAGCTTCCAGTGCAAC-3 ′	122
	R:5 ′ -TGGAACACGTAAAGACCCCT -3 ′	
	FAM-probe 5-GTGGGCCTGCGACAAC-3	
	VIC-probe 5 ′ -CTGCAACAACGACCCCG-3	
LDL-R rs72658860	F:5 ′-CAGGGACCAACGAATGCTTG-3 ′	129
	R:5 ′-AATCACCTTCGCATCTTCGC-3 ′	
	FAM-probe 5 ′ -ACAACGGCGGCTGTTC-3 ′	
	VIC-probe 5 ′-AACGGCAGCTGTTCCC-3 ′	

probes used in the allelic discrimination, as shown in (Table 1).

Real Time PCR was conducted in a complete quantity of 25 µl containing 0.5 µl of forward and reverse primers, 0.5 µl for each FAM and VIC sample, 12.5 µl TaqMan master mix, 6.5 µl PCR nuclease free water, and 4 µl Genomic DNA. Real-time PCR is original denaturation at 95C for 5 min accompanied by 5 denaturation cycles at 95C for 20 s, annealing at 60C for 30 s.

2.4. Data analysis

Data analysis was conducted by using Statistical Package for the Social Sciences (SPSS) for Windows, version 22 (SPSS Inc. Chicago, Illinois, United States). The Shapiro-Wilks test for normality was used to determine if the studied parameters followed a gaussian distribution. Categorical variables were expressed as proportions. The proportions were compared by using the Chi-square test (χ^2) and Fisher's exact test. Data were expressed as mean ± standard deviation (SD) for continuous variables. Differences between groups were analyzed by student's t-test preceded by Levene's test for equality of variances. The Games-Howell and Scheffee Post Hoc tests for multiple comparison was applied after ANOVA tests. The genotype frequencies of each SNP were measured for each locus and tested for Hardy-Weinberg equilibrium (HWE).

3. Results and discussion

The clinical and demographic characteristics of the patient subgroups, summarized in (Table 2). The results showed there were no statistically significant differences in the two subgroups of ACAD in all parameters of demographic characteristics and lipid profiles.

Table 2
Clinical and demographic characteristics of the study groups.

Parameters	Patients with 20 mg (n = 52)	Patients with 40 mg (n = 48)	*P* value
Age (year)[a]	47.66 ± 9.25	48.08 ± 7.26	(NS)
Weight (kg)[a]	79.04 ± 11.42	81.41 ± 12.47	(NS)
Height (cm)[a]	171.91 ± 9.00	170.37 ± 8.38	(NS)
BMI (kg/m^2)[a]	27.96 ± 4.58	28.05 ± 3.70	(NS)
Systolic pressure (mmHg)[a]	12.75 ± 4.12	11.21 ± 2.66	(NS)
Diastolic pressure (mmHg)[a]	7.05 ± 1.70	8.12 ± 3.29	(NS)
TC (mg/dl)	292.82 ± 81.42	322.90 ± 107.56	(NS)
TG (mg/dl)	160.36 ± 38.13	164.92 ± 59.78	(NS)
HDL-C (mg/dl)	49.25 ± 5.00	48.68 ± 5.16	(NS)
LDL-C (mg/dl)	212.52 ± 70.72	245.51 ± 103.58	(NS)
VLDL (mg/dl)	32.07 ± 7.62	32.98 ± 11.95	(NS)

Data were expressed as counts with percentages in parentheses or mean ± SD.
Statistical analysis performed by the Chi-squared test; NS, no significant differences.
[a] Statistical analysis was performed by student t-test.

Table (3A)
Numbers and percentage frequencies (Observed and Expected) of *LDL-R* gene (rs200727689 SNP) genotype and their Hardy-Weinberg equilibrium (HWE) in patients and controls.

Genotype	Patient (No. = 100)		Control (No. = 100)	
	Observed	Expected	Observed	Expected
	No (%)	No (%)	No (%)	No (%)
GG	35 (35)	32.49 (32.49)	59 (59)	58.52 (58.52)
GA	44 (44)	49.02 (49.02)	35 (35)	35.96 (35.96)
AA	21 (21)	18.49 (18.49)	6 (6)	5.52 (5.52)
HWE Analysis	$\chi^2 = 1.05$, DF = 1, P > 0.05		$\chi^2 = 0.07$, DF = 1, P > 0.05	

1 degree of freedom (d.f) for Chi-squared distribution.

Table (3B)
Comparison of the Genotype and Allele Frequencies detected by Hardy-Weinberg equilibrium law of *LDL-R* gene polymorphism rs200727689 between Patient group and control group.

Groups		*LDL-R* gene at position +502 (dbSNP-ID:rs200727689)				
		Genotypes			Alleles	
		GG	GA	AA	G	A
Patients (No. = 100)	No.	35	44	21	114	86
	%	35%	44%	21%	57%	43%
Controls (No. = 100)	No.	59	35	6	153	47
	%	59%	35%	6%	76.5%	23.5%
OR		0.37	1.46	4.16	0.41	2.46
EF or PF		0.37	0.14	0.16	0.45	0.26
P		0.001	0.247	0.003	0.000	0.000
95% (C.I.)		0.21–0.66	0.83–2.57	1.61–10.77	0.27–0.63	1.60–3.77

(OR): Odds Ratio (EF)Etiological Fraction (PF)Preventive Fraction; *P*: Fisher's Exact Probability.

Obesity, hypertension, diabetes mellitus, physical inactivity, and smoking are all contributors to the development of atherosclerotic coronary vascular disease [8].

3.1. Genotype and allele frequency distributions

This study examined the two *LDL-R* gene polymorphisms, the rs200727689 (G > A) SNP presented in the (ligand-binding domain) at exon 4 and the rs72658860 (G > A) SNP presented in the epidermal growth factor precursor as domain (EGFP) at exon 7 to determine the prospective impact of atherosclerosis susceptibility by raising or reducing atherosclerosis. The presence of genetic polymorphism was investigated by using Real-Time PCR (RT-PCR) thermal profile of allelic discrimination techniques.

Table (4A)
Numbers and percentage frequencies (Observed and Expected) of *LDL-R* gene (rs72658860 SNP) genotype and their Hardy-Weinberg equilibrium (HWE) in patients and controls.

Genotype	Patient (No. = 100)		Control (No. = 100)	
	Observed	Expected	Observed	Expected
	No (%)	No (%)	No (%)	No (%)
GG	30 (30)	35.4 (35.4)	57 (57)	58.52 (58.52)
GA	59 (59)	48.2 (48.2)	39 (39)	35.96 (35.96)
AA	11 (11)	16.4 (16.4)	4 (4)	5.52 (5.52)
HWE Analysis	$\chi^2 = 5.03$, DF = 1, P < 0.05		$\chi^2 = 1.05$, DF = 1, P > 0.05	

1 degree of freedom (d.f) for Chi-squared distribution.

Table (4B)
Comparison of the Genotype and Allele Frequencies detected by Hardy-Weinberg equilibrium law of *LDL-R* gene polymorphism rs72658860 between Patient group and control group.

Groups		*LDL-R* gene at position +970 (dbSNP-ID: rs72658860)				
		Genotypes			Alleles	
		GG	GA	AA	G	A
Patients (No. = 100)	No.	30	59	11	119	81
	%	30%	59%	11%	59.5%	40.5%
Controls (No. = 100)	No.	57	39	4	153	47
	%	57%	39%	4%	76.5%	23.5%
(OR)		0.32	2.25	2.97	0.45	2.22
EF or PF		0.39	0.33	0.073	0.42	0.22
P		0.0001	0.007	0.105	0.0003	0.0003
95% (C.I.)		0.18–0.58	1.28–3.95	0.92–9.60	0.29–0.69	1.44–3.41

(OR): Odds Ratio (EF) Etiological Fraction (PF) Preventive Fraction; *P*: Fisher's Exact Probability.

Table (5A)
Impact of *LDL-R* polymorphism rs200727689 on the lipid profile of the control group.

Parameters	Genotype (mean + SD)			
	G/G (n = 59)	G/A (n = 35)	A/A (n = 6)	P value[‡]
TC (mg/dl)	136.82 ± 20.24	135.40 ± 18.36	175.23 ± 27.94[b,c]	0.000
TG (mg/dl)	116.19 ± 20.27	106.17 ± 9.79[a]	113.82 ± 22.26	0.03
HDL (mg/dl)	52.81 ± 9.84	50.93 ± 8.48	49.79 ± 9.04	0.54
LDL (mg/dl)	61.52 ± 19.01	63.23 ± 18.85	102.67 ± 20.94[b,c]	0.000
VLDL (mg/dl)	23.11 ± 4.02	21.44 ± 2.08	22.76 ± 4.45	0.09

[‡] ANOVA significance test (2-tailed).
[a] *P*< 0.05 GG group *vs*. GA group.
[b] *P*< 0.01 GG group *vs*. AA group.
[c] *P*< 0.01 GA group *vs*. AA group.

3.1.1. LDL-R gene SNP at +502 position (rs200727689)

The *LDL-R* gene SNP at position +502 ($LDLR_{+502}$) was presented with three genotypes (GG, GA and AA) that were corresponding to two alleles (*G* and *A*). Hardy-Weinberg equilibrium (HWE) analysis showed that there was no significant difference in patient or control frequencies between the observed and expected genotype frequencies (Table 3A).

Among these genotypes and alleles, it has been found that the mutant allele homozygous (AA) has a significant increase in the percentage of patients compared with the control group (21% vs. 6%) (P = 0.003) and 4.16 (95% CI: 1.61–10.77; EF = 0.16) respectively. It was also observed that *A* allele showed a significant increase frequency in patients (43% *vs* 23.5%; OR = 2.46; 95% C.I: 1.60–3.77) (p = 0.000). In the state of homozygosity

Table 5(B)
The lipid profile and atorvastatin therapy response to (20 mg/day) in the patients with atherosclerotic coronary artery disease, according to the low-density lipoprotein receptor (LDL-R) rs200727689 polymorphism.

Parameters	Genotype (mean + SD)			
	G/G (n = 21)	G/A (n = 22)	A/A (n = 9)	P value[‡]
TC (mg/dl)	300.0 ± 73.99	328.03 ± 123.01	266.90 ± 103.60	0.56 (NS)
TG (mg/dl)	161.79 ± 56.88	176.35 ± 34.47	169.97 ± 42.72	0.79 (NS)
HDL (mg/dl)	47.00 ± 6.81	54.37 ± 6.23	45.22 ± 16.30	0.19 (NS)
LDL (mg/dl)	250.01 ± 62.52	274.12 ± 103.84	198.33 ± 100.0	0.22 (NS)
VLDL (mg/dl)	32.35 ± 11.37	35.26 ± 6.88	34.00 ± 8.55	0.79 (NS)

NS, no significance differences.
[‡] ANOVA test (2-tailed).

Table 5(C)
The lipid profile and atorvastatin therapy response to (40 mg/day) in the patients with atherosclerotic coronary artery disease, according to the low-density lipoprotein receptor (LDL-R) rs200727689 polymorphism.

Parameters	Genotype (mean + SD)			
	G/G (n = 14)	G/A (n = 22)	A/A (n = 12)	P value‡
TC (mg/dl)	293.61 ± 79.94	282.46 ± 74.91	394.24 ± 132.66[c]	0.047
TG (mg/dl)	164.38 ± 37.36	148.20 ± 30.80	179.07 ± 86.07	0.47
HDL (mg/dl)	46.66 ± 9.30	49.00 ± 9.13	45.50 ± 12.08	0.71
LDL (mg/dl)	234.02 ± 26.16	203.86 ± 52.44	312.91 ± 122.26[c]	0.01
VLDL (mg/dl)	32.87 ± 7.47	29.63 ± 6.17	35.80 ± 17.21	0.47

‡ ANOVA significance test (2-tailed).
[c] $P^{<}$ 0.05 GA group *vs.* AA group.

Table 6(A)
Impact of *LDL-R* polymorphism rs72658860 on the lipid profile of the control group.

Parameters	Genotype (mean + SD)			
	G/G (n = 57)	G/A (n = 39)	A/A (n = 4)	P value‡
TC (mg/dl)	140.66 ± 24.03	134.69 ± 18.02	155.62 ± 20.27	0.13 (NS)
TG (mg/dl)	113.40 ± 18.53	110.99 ± 18.17	107.93 ± 6.01	0.73 (NS)
HDL (mg/dl)	51.85 ± 9.77	52.17 ± 9.20	51.83 ± 9.47	0.98 (NS)
LDL (mg/dl)	66.13 ± 22.80	60.32 ± 18.49	82.20 ± 22.31	0.11 (NS)
VLDL (mg/dl)	22.68 ± 3.70	22.19 ± 3.63	21.58 ± 1.20	0.73 (NS)

NS, no significance differences.
‡ ANOVA test (2-tailed).

of the wild type allele (GG), there was a decrease significant difference observed between patients and control groups (35%*vs* 59%) (p = 0.001) and the association OR was 0.37 (95% CI: 0.21−0.66; PF = 0.37), the *G* allele showed a significant difference between patients and control groups respectively (57 v 76.5%; OR = 0.41; 95C.I: 0.27−0.63) p = 0.000. The heterozygous (GA) has no significant difference between the two groups (44% vs 35%) OR = 1.46 (95% CI: 0.83−2.57; EF = 0.14) P= (0.247) (Table 3B).

3.1.2. *LDL-R gene SNP at +970 position (rs72658860)*

The *LDL-R* gene SNP at position +970 ($LDLR_{+970}$) was presented with three genotypes (GG, GA and AA) that were corresponding to two alleles (*G* and *A*) in two investigated groups. There was a significant difference between the observed and expected genotype frequencies in patients, no significant difference between the observed and expected genotype frequencies in controls; therefore the Hardy-Weinberg equilibrium HWE was established (Table 4A).

Table 6(B)
The lipid profile and atorvastatin therapy response to (20 mg/day) in the patients with atherosclerotic coronary artery disease, according to the low-density lipoprotein receptor (LDL-R) rs72658860 polymorphism.

Parameters	Genotype (mean + SD)			
	G/G (n = 7)	G/A (n = 18)	A/A (n = 4)	P value‡
TC (mg/dl)	277.30 ± 88.70	361.46 ± 98.62	256.45 ± 64.05[c]	0.049
TG (mg/dl)	151.80 ± 30.71	152.16 ± 34.64	148.66 ± 39.08	0.97
HDL (mg/dl)	52.71 ± 7.06	48.55 ± 10.81	52.25 ± 8.73	0.57
LDL (mg/dl)	183.54 ± 47.60	279.50 ± 60.00[a]	179.97 ± 43.30[c]	0.000
VLDL (mg/dl)	30.36 ± 6.14	30.43 ± 6.92	29.73 ± 7.81	0.97

‡ ANOVA significance test (2-tailed).
[a] $P^{<}$ 0.05 GG group *vs.* GA group.
[c] $P^{<}$ 0.05 GA group *vs.* AA group.

Table 6(C)
The lipid profile and atorvastatin therapy response to (40 mg/day) in the patients with atherosclerotic coronary artery disease, according to the low-density lipoprotein receptor (LDL-R) rs72658860 polymorphism.

Parameters	Genotype (mean + SD)			
	G/G (n = 9)	G/A (n = 19)	A/A (n = 3)	P value‡
TC (mg/dl)	313.33 ± 119.65	328.87 ± 68.86	356.40 ± 56.22	0.62 (NS)
TG (mg/dl)	161.20 ± 30.97	158.12 ± 37.11	151.60 ± 48.40	0.87 (NS)
HDL (mg/dl)	48.60 ± 5.15	47.92 ± 4.68	53.40 ± 5.68	0.08 (NS)
LDL (mg/dl)	234.02 ± 49.62	250.36 ± 66.89	274.00 ± 66.63	0.43 (NS)
VLDL (mg/dl)	32.24 ± 6.18	31.62 ± 7.42	30.32 ± 9.68	0.87 (NS)

NS, no significance differences.
‡ ANOVA test (2-tailed).

There was no significant difference in the distribution of homozygous wild type (GG) between patients and controls (30% vs 57%; OR = 0.32; 95% C.I: 0.18−0.58; PF = 0.39). In addition, there were no significant differences observed in the mutant allele (AA) in comparison with two groups (11% vs 4%; p = 0.007; OR = 2.97; 95% C.I: 0.92−9.60; EF = 0.073). Comparing patients to control revealed that *G* and *A* allele frequency were significant differences, the *G* allele decreases in patients by comparing with control (59.5% *vs* 76.5%; P = 0.0003; OR = 0.45; 95% C.I: 0.29−0.69) and the *A* allele increase in patients compared with control group (40.5% *vs* 23.5%; P = 0.0003; OR = 2.22; 95% C.I: 1.44−3.41). The heterozygous genotype has a significant difference observed between the two groups (59% vs 39%; P = 0.007; OR = 2.25; C.I: 1.28−3.95; EF = 0.33) (Table 4B).

It is possible to consider *A* as a predisposing allele for ACAD, while *G* allele may have a protective effect against the development of disease. These findings came in line with the presented results from Ref. [9] in Portuguese atherosclerotic coronary artery disease patients, in which significant variation between patients and controls was observed in genotype distribution of rs200727689. An earlier study related to these results [10], demonstrated pathogenicity of the rs72658860 SNP and highly distributed in patients with hypercholesterol in Mexican individuals, and [11] showed the predisposing effect of cardiovascular disease occurred with relatively high frequencies in specific parts of the Netherlands.

3.2. The pharmacogenetic effect of single nucleotide polymorphisms (SNPs) on lipid-lowering response to atrovastatins

Atorvastatin controls elevated levels of cholesterol and reduces the risk of heart disease; therefore, studying the association between susceptible gene polymorphism and lipid-reducing efficacy is a great benefit to the clinical application of atorvastatin [12]. Recently, a major focus has been on the genetic factor as a major contributor to a drug response, but a continuum of different responses across treated persons has appeared when an equivalent dosage is used [13]. Atorvastatin is one of those whose drug effectiveness is affected by both genetic and environmental factors. Thus, studying the association between lipid-lowering effectiveness and susceptible gene polymorphism is very important for clinical application of atorvastatin [12].

3.2.1. Pharmacogenetics effect of rs200727689

The levels of serum lipid in control and patients that treated with (20 mg and 40 mg) according to rs200727689 genotype are shown in Table (5A) (5B) and (5C) respectively. In the control group, the plasma level of total cholesterol was significantly higher in the AA genotype than in the GG and GA genotype (175.23 ± 27.94 vs 136.82 ± 20.24) and (175.23 ± 27.94 vs 135.40 ± 18.36) P= (0.000), also the plasma LDL-C was significantly higher in AA genotype than in the GG and GA genotype (102.67 ± 20.94 *vs* 61.52 ± 19.01) and (102.67 ± 20.94 *vs* 63.23 ± 18.85) P= (0.000). The plasma level of triglyceride was significantly decreased in GA genotype compared with GG genotype (106.17 ± 9.79 *vs* 116.19 ± 20.27) P= (0.03). Other parameters such as HDL and VLDL were not significantly different between various genotypes in the control group.

The results showed no statistically significant differences in patients treated with atorvastatin 20 mg in all parameters (TC, TG, HDL, LDL, and VLDL) p > 0.05, but the result in AA genotype observed reduction in TC (277.55 ± 108.38 *vs* 326.99 ± 63.56) and LDL-C (198.33 ± 100.0 *vs* 250.01 ± 62.52) concentration in sera of patients treated with 20 mg atorvastatin compare with GG genotype.

Interestingly, the minor allele (*A*) of rs200727689 has been linked to an improved response to 20 mg atorvastatin treatment.

In addition, *A* allele homozygous carriers had the highest serum level of total and LDL cholesterol in patients treated with 40 mg atorvastatin compared with GA genotype (394.24 ± 132.66 vs 282.46 ± 74.91) P = 0.047 and (312.91 ± 122.26 vs 203.86 ± 52.44) P= (0.01) respectively. There were no significant differences in other parameters in patients with 40 mg atorvastatin between various genotypes.

3.2.2. *Pharmacogenetics effect of rs72658860*

The serum lipid levels in control and patients treated with (20 mg and 40 mg) subgroup participants according to rs72658860 genotype are shown in table (6A) (6B) and (6C) respectively. In the control group, there were no significant differences observed in all parameters of the lipid profile P > 0.05 between various genotypes, the result was a small increase in AA genotype TC and LDL-C serum levels compared with GG and GA.

The results in patients treated with 20 mg atorvastatin observed decrease in TC in GG and AA genotypes but significantly occurred in AA compared with GA genotype (256.45 ± 64.05 vs 361.46 ± 98.62) P = 0.049, also a significant reduction in LDL-C serum level between GG and GA genotypes (183.54 ± 47.60 vs 279.50 ± 60.00), and between AA and GA genotypes (179.97 ± 43.30 vs 279.50 ± 60.00), there were no significant differences observed in other parameters between various genotypes.

The serum lipid level in patients treated with 40 mg atorvastatin was observed there were no significant differences in all biochemical parameters with regard to rs72658860 SNP.

The association between homozygous carriers of *A* allele and response to atorvastatin medication has been revealed, according to this study. Despite the dose of 40 mg/day atorvastatin treatment, it should confirm that treatment with 40 mg/day of atorvastatin had been inadequate to reduce the levels of lipid to recommended target values and improvement seen in 20 mg/day of atorvastatin in both TC and LDL-C.

In relation to pharmacogenetics of atorvastatin therapy, one of the variants showing more reproducibility with treatment response is the AA genotype in 20 mg patients compared to 40 mg in rs200727689, this allele changed from highly hydrophilic polar amino acid (aspartic acid) to polar amino acid (asparagine) of exon 4 [14]. It was found that the AA genotype of rs72658860 was significantly associated with the response to 20 mg atorvastatin; this allele changed the polar amino acid glycine to serine [9].

Atorvastatin is a relatively lipophilic compound and is the most effective in reducing low-density lipoprotein cholesterol, providing a rational basis for its use in low-dose clinical practice [15]. The lipophilic statin appears to have a higher level of non-hepatic cell exposure and passive non-selective access to both hepatic and non-hepatic tissues [16,17]. The hypolipedimic activity of statins is modified by genetic factors. There have been found several gene polymorphisms that affect statin activity, however pharmacogenetic variability may also be a risk factor for adverse drug response [18].

Studies show that atorvastatin reduces total blood cholesterol and LDL-cholesterol in a linear dose-related manner over the commonly prescribed dose range; the effect was higher at lower doses than at higher doses [19]. The results suggested by Ref. [20] that the hypolipidemic activity manifested at the dose-dependent of atorvastatin (20 mg/day), which characterized by a decrease in both cholesterol levels and the atherogenic index. Treatment with low-dose atorvastatin appears to be safe and well tolerated in early-stage patients, reducing total cholesterol, LDL-C, oxLDL, and therefore helping to improve vascular functions [21].

Likewise, the result was confirmed by Ref. [22] who observed a significant reduction in atorvastatin 20 mg/day in four surrogate markers following administration of atorvastatin in both patients and control groups, and [23] suggested that the aggressive treatment with statins is not more effective for controlling lipid levels and cannot prevent future ischemic events. Such differences with [24] who suggests the intensive lipid-lowering therapy with 80 mg of atorvastatin per day in patients with stable (CHD) provides significant clinical benefits beyond that afforded by treatment with 10 mg of atorvastatin per day. It has been reported that the response to statins has been affected by the mutation class in reducing LDL and apolipoprotein B. After treatment with statins, the patient with class V mutations in which the internalized LDL particles cannot be released into the endosomes shows a high reduction in LDL and apolipoprotein B levels [25]. Furthermore, the answer differs among individuals, possibly due to factors such as gender, ethnicity or genetic composition patients of Asian ancestry have greater lipid reduction activity compared with European ancestry at lower doses of atorvastatin and rosuvastatin [26,27].

In previous studies, two SNPs in the LDL-R gene (rs1433099) and (rs5925) have been identified to be associated with lipid-lowering. The first polymorphism has a significant association between lower levels of

LDL-C and cardiovascular disease with pravastatin-treated patients with coronary heart disease [28]. The latter SNP seems capable to significantly influence of the LDL-C response to pravastatin in patients with hypercholesterolemia [29]. From the previously available data [2], concluded that carriers of the E4 (Ɛ 4) allele of *the ApoE gene* tend to have a greater percentage reduction in LDL cholesterol in response to statin therapy than participants carrying the wild-type ApoE allele (E3 or Ɛ 3).

4. Conclusion

Finally, it may be concluded that, this study is the first to identify the LDL-R-gene variants related to the dose-response of atorvastatin to lower the TC and LDL-C serum level with a specific genotypes. The study showed that the two SNPs of *LDL-R* gene (rs200727689; rs72658860) affected the atorvastatin effectiveness in all the treated patients and revealed an effective response to 20 mg of atorvastatin in the Iraqi population in reduction of TC and LDL-C with AA genotype and it was observed in rs72658860. Whereas the toxicity and efficacy are related to the statin dose, carriers of these SNPs may be benefit more from the high potency of 20 mg atorvastatin to achieve aggressive aims; these findings can provide a basis for customized therapy for cardiovascular disease patients. In order to confirm our findings, future studies with large sample sizes are required.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee. The ethics committee of the Iraqi Ministry of Health, the Iraqi center of heart disease at Surgical specialist (Ghazi Al -Hariri) Hospital/ Baghdad, Iraq approved the study according to the reference number: 1278/ 16-5-2018.

Funding

None.

Competing interests

None.

Acknowledgements

We kindly thank all the individuals for their cooperation and participation in the study and the cooperation of the medical staff at Surgical specialist (Ghazi Al -Hariri) Hospital/ Baghdad, Iraq.

References

[1] J. Poss, F. Custodis, C. Werner, O. Weingartner, M. Bohm, U. Laufs, Cardiovascular disease and dyslipidemia: beyond LDL, Curr. Pharmaceut. Des. 9 (2011) 861–870.
[2] K. Kajinami, H. Akao, E. Polisecki, E. Schaefer, Pharmacogenomics of statin responsiveness, Am. J. Cardiol. 9 (2005) 65–70.
[3] P.E. Miller, S.M. Seth, Approach to statin use in 2016: an update, Curr. Atherosclerosis Rep. 18 (2016) 20.
[4] K. Kajinami, N. Takekoshi, M.E. Brousseau, E.J. Schaefer, Pharmacogenetics of HMG-CoA reductase inhibitors: exploring the potential for genotype-based individualization of coronary heart disease management, Atherosclerosis 2 (2004) 219–234.
[5] T.P. Aneesh, S. Sonal, J. Asha, C. Lekshmi, M.Z. Subin, Pharmacogenomics: the right drug to the right person, J. Clin. Med. Res. 1 (2009) 191.
[6] N. Soko, D. Collet, R. Raj, K. Gerard, M. Collen, Pharmacokinetics of rosuvastatin in 30 healthy Zimbabwean individuals of African ancestry, Br. J. Clin. Pharmacol. 82 (2016) 326.
[7] C. Liu, Y. Jing, H. Wei, Z. Qi, S. Xiaoming, L. Xia, L. Feng, L. Xiaokun, Polymorphisms in ApoB gene are associated with risk of myocardial infarction and serum ApoB levels in a Chinese population, Int. J. Clin. Exp. Med. 8 (2015) 16571.
[8] J.C. LaRosa, C.D. Brown, Cardiovascular risk factors in minorities, Am. J. Med. 12 (2005) 1314–1322.
[9] A.M. Medeiros, A.C. Alves, V. Francisco, M. Bourbon, Update of the Portuguese familial hypercholesterolaemia study, Atherosclerosis 2 (2010) 553–558.
[10] G. Vaca, A. Vàzquez, M.T. Magaña, M.L. Ramìrez, I.P. Dàvalos, E. Martìnez, B. Marìn, G. Carrillo, Mutational analysis of the LDL receptor and APOB genes in Mexican individuals with autosomal dominant hypercholesterolemia, Atherosclerosis 2 (2011) 391–396.
[11] S.W. Fouchier, J.C. Defesche, M.A. Umans-Eckenhausen, J.J. Kastelein, The molecular basis of familial hypercholesterolemia in The Netherlands, Hum. Genet. 6 (2011) 602–615.
[12] L. Lesko, J. Lawrence, Z. Issam, DNA, drugs and chariots: on a decade of pharmacogenomics at the US FDA, Pharmacogenomics 11 (2010) 507–512.
[13] J. Lagos, T.Z. Jenny, R. Alexy, S. Luis, APOE polymorphisms contribute to reduced atorvastatin response in Chilean Amerindian subjects, Int. J. Mol. Sci. 16 (2015) 7890–7899.
[14] R. Durst, U.K. Ibe, S. Shpitzen, D. Schurr, O. Eliav, M. Futema, R. Whittall, A. Szalat, V. Meiner, H. Knobler, D. Gavish, Molecular genetics of familial hypercholesterolemia in Israel–revisited, Atherosclerosis 257 (2017) 55–63.
[15] M. Schachter, Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update, Fund. Clin. Pharmacol. 19 (2005) 117–125.
[16] C. Fong, Statins in therapy: understanding their hydrophilicity, lipophilicity, binding to 3-hydroxy-3-methylglutaryl-CoA reductase, ability to cross the blood brain barrier and metabolic stability based on electrostatic molecular orbital studies, Eur. J. Med. Chem. 85 (2014) 661–674.
[17] J. Spence, G. Dresser, Overcoming challenges with statin therapy, J. Am. Heart Assoc. 5 (2016) 497–510.

[18] D. Kotlęga, P. Nowacki, M. Białecka, M. Kurzawski, M. Droździk, S. Ciećwież, Association between CRP gene polymorphism 717A/G, C-reactive protein and neurological deficit in ischemic stroke, J. Clin. Neurosci. 21 (2014) 574−577.

[19] S. Adams, P.T. Michael, M.W. James, Atorvastatin for lowering lipids, Cochrane Database Syst. Rev. 3 (2015).

[20] O. Barbarash, O. Gruzdeva, E. Uchasova, E. Belik, Y. Dyleva, V. Karetnikova, Dose-dependent effects of atorvastatin on myocardial infarction, Drug Des. Dev. Ther. 9 (2015) 3361.

[21] T. Stojakovic, T. Claudel, C. Putz-Bankuti, G. Fauler, H. Scharnagl, M. Wagner, H. Sourij, R.E. Stauber, K. Winkler, W. März, T. Wascher, Low-dose atorvastatin improves dyslipidemia and vascular function in patients with primary biliary cirrhosis after one year of treatment, Atherosclerosis 209 (2010) 178−183.

[22] R. Sadeghi, M. Asadpour-Piranfar, M. Asadollahi, M. Taherkhani, F. Baseri, The effects of different doses of atorvastatin on serum lipid profile, glycemic control, and liver enzymes in patients with ischemic cerebrovascular accident, ARYA Atherosclerosis 10 (2014) 298.

[23] G.G. Schwartz, Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) Study Investigators. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes; the MIRACL study; a randomized controlled trial, Jama 285 (2001) 1711−1718.

[24] J.C. LaRosa, S.M. Grundy, D.D. Waters, C. Shear, P. Barter, J.C. Fruchart, A.M. Gotto, H. Greten, J.J. Kastelein, J. Shepherd, N.K. Wenger, Intensive lipid lowering with atorvastatin in patients with stable coronary disease, N. Engl. J. Med. 352 (2005) 1425−1435.

[25] G. Miltiadous, S. Xenophontos, E. Bairaktari, M. Ganotakis, M. Cariolou, M. Elisaf, Genetic and environmental factors affecting the response to statin therapy in patients with molecularly defined familial hypercholesterolaemia, Pharmacogenetics Genom. 15 (2015) 219−225.

[26] K. Ferdinand, Ethnic, gender, and age-related differences in the treatment of dyslipidemia, Am. J. Manag. Care 12 (2006) S400−S404.

[27] Q. Feng, R.A. Wilk, T.M. Baye, Individualized risk for statin-induce mayopathy, Pharmacogenomics 13 (2012) 579−594.

[28] E. Polisecki, M. Hind, M. Nobuyo, P. Inga, R. Michele, D. Alex, I.F. McMahon, Genetic variation at the LDL receptor and HMG-CoA reductase gene loci, lipid levels, statin response, and cardiovascular disease incidence in PROSPER, Atherosclerosis 200 (2008) 109−114.

[29] C. Lahoz, P. Rocío, M.M. Jose, L. Fernando, F.M. María, T. Manuel, P. Xavier, RAP Study Group, "Baseline levels of low-density lipoprotein cholesterol and lipoprotein (a) and the AvaII polymorphism of the low-density lipoprotein receptor gene influence the response of low-density lipoprotein cholesterol to pravastatin treatment, Metabolism 54 (2005) 741−747.