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SNP of HMGCR and Apo E genes and their impact in response to statin therapy in hypercholesterolemic and hypertriglyceridemic patients in Pakistan

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Abstract: Coronary artery disease (CAD) and the problems associated with it are the most prominent causes of death in the whole world. Statins are accustomed to lower lipid levels in CAD patients. The target of this study was to analyze whether or not common variations in HMGCoA Reductase (HMGCR) and Apolipoprotein E (ApoE) genes are responsible for metabolism of lipid and statin that modify the impact of statins on serum level of lipids and lipoprotein concentrations in Coronary heart disease patients. One hundred CAD patients were registered for the study. At the start of the study biochemical measurements were performed to work out the baseline levels. Patients were treated with twenty mg Lipitor for one month and biochemical measurements were tested again. According to the post-treatment, LDL-c levels, patients were divided into a pair of group as non-responders and responders, independently. The information concerning the risk factors like smoking, alcohol consumption etc. was conjointly obtained. DNA was extracted from peripheral blood. The presence of rs17244841 and rs17238540 mutations in HMGCR and ε2, ε3 and ε4 variants of ApoE were settled by performing RT-PCR. Results were assessed statistically. HMGCR mutations were principally found in responders and ε4 variant of ApoE was principally found in non-responders. It was found that the presence of HMGCR mutations causes a big reduction in total cholesterol and LDL-c levels. Conjointly, the presence of ε2 variant of Apo E causes a statistically vital increase in triglyceride levels. Our findings should be investigated by different researchers to clarify the mechanism.

Keywords: Single nucleotide polymorphism, low density lipoproteins, cholesterol, statin, fibrate.

INTRODUCTION

Coronary heart disease is the most prominent reason for deaths globally, and was foreseen in most of the developing nations as the most important reason for morbidity and mortality by 2020 worldwide (Celermajer *et al*. 2012). One of the most important risk factor of Coronary heart disease is dyslipidemia (Shanmugasundaram *et al.* 2010), a disease of lipid and conjugated protein metabolism (Radovica *et al*. 2014). Dyslipidemia is characterized by the enhanced total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), or triglyceride (TG) concentration, or reduced high-density lipoprotein-cholesterol (HDL-C) concentration within the blood (Radovica *et al*. 2014).

Several genetic loci that are related to the levels of various lipids within blood have been found in the current human genetic and genome wide association studies (GWAS). A lot of lipids within the blood are affected by these genetic variants (Abifadel *et al*. 2003; Kathiresan *et al*. 2008; Kooner *et al.* 2008; Wallace *et al*. 2008; Willer *et al*. 2008; Chasman *et al*. 2009; Deo *et al*. 2009; Teslovich *et al.* 2010; Kim *et al*. 2011; Sarzynski *et al*. 2011; Inouye *et al*. 2012).

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Statins or the β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) enzyme inhibitors are among the foremost pharmaceuticals use to decrease the chance of cardiovascular incidents globally (Peters *et al*, 2011). Statins reduce LDL-c with effectively and are responsible for reducing clinical issues in each primary and secondary interference of coronary heart disease (Fiegenbaum *et al*, 2005). Statins prohibit internal cholesterol production by competitive inhibition of HMGCR, the catalyst that activates transformation of HMG-CoA to mevalonate, that is the early rate-limiting step in the synthesis of cholesterol. By reducing intracellular cholesterol production, statin treatment results in up regulation of low-density lipoprotein (LDL) receptors, leading to increased plasma clearance of LDL, primarily by the liver. Additionally, statin will scale back the very low-density lipoprotein (VLDL), LDL and Apo B-containing lipoproteins secreted by the liver (Mangravite *et al*, 2006). Typically statin treatment is related to a LDL-c lowering up to fifty five persont and a dicline of cardiovascular incidents by 20-30% (Postmus *et al*, 2012).

Increase in high-density lipoprotein cholesterol (HDLc) and reductions in plasma triglyceride levels are the other consequences of potential clinical significance (Mangravite *et al,* 2006). Statins are extensively

prescribed medications that forestall incident and repeated CAD events primarily through the reduction of LDL-c. The goal of lipid-lowering therapy is to scale back the LDL-c cholesterol to 100mg/dL in individuals with high risk associate in a secondary goal of 70mg/dL for individuals at highest risk. As a result of these aggressive recommendations, a considerably higher proportion (40%) of patients treated outside of clinical trials stay higher than their suggested LDL-c goal (Voora *et al*, 2008). Non-genetic and environmental factors, like baseline plasma concentrations of LDL-c age, physical activity, racial ancestry, smoking standing, diet and body weight are responsible for the variability in response to statin therapy. Baseline LDL-c levels are also controlled by genetic factors (Krauss *et al* 2008). Most of the recent studies showed that the possible contributors to the variation in statin therapy response are the genetic factors (Chasman *et al,* 2004, Thompson *et al*, 2005).

HMGCR plays a crucial role in hepatic regulation of plasma cholesterol that makes it a principal candidate gene to understand the genetic sequence variation related to each basal and statin-responsive lipid and lipoprotein concentrations (Krauss *et al*., 2008). So HMGCR gene, coding HMG-CoA reductase, is a very important candidate factor for the pharmacogenomics of statins (Peters *et al*, 2011). HMGCR spans regarding 24200 base pairs on chromosome 5q13.3. rs17244841 (also reported as SNP12) A>T base substitution and rs17238540 (also reported as SNP29) T>G base substitution are determine in intron 5 and intron 18, respectively. (Chasman *et al*, 2004, Thompson *et al*, 2009). These 2 variations were noted to be related to LDL-c response during a cohort study with lipid-lowering medication (Chasman *et al,* 2004).

Other cholesterol pathway connected genes may be of importance for statin drug responsiveness (Peters *et al,* 2011). One in all the foremost deeply studied genetic factors is that the Apo E genotype. Apo E is one of the protein part of chylomicrons, remnant particles, very low density lipoproteins (VLDL) and high density lipoproteins (HDL) that serves as a substance of ligand for their receptor-mediated catabolism through low density lipoproteins (LDL) and therefore the ApoE receptor (Christidis *et al*, 2006). ApoE may be a 34-kDa glycosylated polymorphic protein, its gene islocated on chromosom 19 (Saidi *et al,* 2007). Three common alleles of Apo E known as ε2, ε3, and ε4 are due to the two point mutations in the fourth exon of the gene, committal to writing for the three most prominent Apo E isoforms (Christidis *et al*, 2006). ε2, ε3, and ε4 variants of Apo E correlates at rs429358 and rs7412 for Apo E SNP haplotypes T-T, T-C, and C-C, respectively, (Deshmukh *et al*, 2012).Practically, the association of Apo ε2 alleles with decreased and ε4 alleles commonly with increased total cholesterol and LDL-c levels has proved that lipid profile is affected by Apo E alleles and genotypes (Yamauchi *et al*, 1999, Luc *et al,* 1994). Conjointly it had been found that ε2 carriers cared-for have lower levels of LDL-c total cholesterol compared with ε3 and ε4 carriers throughout statin usage (Christidis *et al*, 2006).

In present study, we tend to study 2 necessary genes associated with lipid balance and with the capability to be genetic indicator of statin responsiveness: Apo E (ε2/ε3/ε4) accountable for the hepatic clearance of triglyceride-rich lipoproteins and HMGCR (rs17244841, rs17238540) that encodes 5-hydroxy-3-methylglutaryl-coenzyme A reductase enzyme and plays a job in hepatic regulation of cholesterol. We tend to conjointly inspect the relation among the genes and environmental factors for example, age, sex, smoking, alcohol intake, and case history in response to lipid-lowering drug therapy.

MATERIALS AND METHODS

In this study, a hundred angiographically confirmed, unrelated CAD patients (44 female, 56 male) attending the Faisalabad Institute of Cardiology Faisalabad, between November 2020 and February 2021 who have not received any lipid lowering therapy so far were enrolled in the study. Baseline and follow up serum lipid levels were measured and statin was prescribed. Individuals with a historical background of stroke, urinary organ diseases, and diabetes mellitus were removed from the experimental study. An overnight baseline blood sample was taken from all the patients after they started on statin therapy (20mg/day) and at one month once beginning medical therapy for calculating plasma lipid and lipoprotein concentrations. The patients were grouped in to two different categories according to their LDL-c levels; fifty patients whose LDL-c levels weren't efficiently reduced (>100mg/dL) and fifty patients whose level of LDL-c was sufficiently decreased (<100mg/DL) were assigned as non-responders and responders respectively.

Genotyping

Every individual supplied 3 ml of blood sample that was stored in sterilized tubes having ethylene diamine tetraacetic acid (EDTA). Whole blood was reserved at -20^{o}C, instantly after collecting samples. Before further processing. By using standard Phenol-Chloroform protocol procedure genomic DNA was isolated from this blood. DNA purity and concentration were determined by Nano Drop spectrophotometer (Thermo Scientific). A 96-well plate was used for PCR amplification in the CFX 96 touch Real-Time PCR System (Bio-Rad, USA). For rs17244841, rs17238540 in HMGCR; and for rs7412 and rs429358 in Apo E for identifying ε2, ε3 and ε4 variants were carried. Primer 3 software was used to design the primer sequences, and the sequences of the forward, reverse and SNP genotyping primers were as follows:

TGCCAACAACAGAACGTT, TGCTAGACGCAAATG GTA and GCCAGTCTAGCAA TACATCATA for rs17244841, GGCGAACATAATGGTGCATA, CGGCA GGTTTATCATGCAGT and GTCGATGATGAATTAT CTGATGC for rs17238540, CAGCATAAGAATCGACT AATACATC, TGGAGCAATCATAGCTATTG and TCGACGAAGCATATTACCATGATC for rs7412 and GAC ACG GCT GTCCAA CGA, GAC CGG GCT TGG TAC ACT GAC and TAC GGC AAT CGT ACT GCC AGC for rs429358. For ApoE genotyping, individuals were grouped accordingly if they had the Apo E2/3 or ApoE2/2 genotype, they were called as ε2 allele carriers; if they were apoE3/3 genotype, were known as ε3 allele carriers; and if they had the apoE4/3 or apoE4/4 genotype, they were named as ε4 allele carriers. The presence of the "T" allele at rs7412 and "T" allele at rs429358 indicates ε2 variant; the presence of the "C" allele at rs7412 and "T" allele at rs429358 express ε3 variant; and the existence of the "C" allele at rs429358 and "C" allele at rs7412 represents ε4 variant (Deshmukh *et al.,* 2012). The reactions were carried out according to the manufacturer's instructions.

Biochemical investigations

Blood specimen, at baseline and one month when the start of the treatment, were taken when 12 hours from the fasting individuals. Standard enzymatic kits (Accurex Pvt. Ltd.) were used to determine the blood levels of triglycerides (TG), HDL-c, LDL-c, Total cholesterol (TC), glucose and alternative relevant biochemical parameters. Weight (kg) divided by height square (m2) was used to measure the body mass index.

STATISTICAL ANALYSIS

The Online Genetic Epidemiology OEGE (http://www. oege.org), a web-based tool was used to analyzed all genotypes and allele frequencies for the chosen SNPs (Santiago Rodriguez, 2009). All genotype frequencies were tested for Hardy–Weinberg equilibrium by using the Pearson goodness-of-fit x2 test with 1° of freedom for biallelic markers. Chi-square (x2) test was used to check the variations in allele/ genotype frequencies among groups. The risk related to a selected gene or genotype in all listed cases was judged by measuring the Odds ratio (OR) and 95% confidence intervals. To analyze all statistical measurements IBM SPSS software system (version 24.0, SPSS Inc., USA), and whole p-values were performed, p values below 0.05 ($p<0.05$) were considered to be statistically significant.

RESULTS

Table1 describe the pre, post-treatment and mean percent decrease in the concentrations of LDL-c and total cholesterol (TC). The plasma concentrations of total cholesterol and LDL-c in responders were significantly decreased when treated with 20 mg per day of statin whereas reductions of LDL-c total cholesterol (TC) were insufficient in non-responders.

According to the significant differences between baseline LDL-c level and post-treatment LDL-c levels, study population was divided into two as non-responders and responders. 50 individuals in which the levels of LDL-c were not sufficiently declined (>100 mg/dl) and 50 individuals in which the levels of LDL-c were sufficiently decreased (<100 mg/Dl) were assigned as non-responders and responders respectively. Table 1 shows LDL-c and total cholesterol levels of groups.

Table 2 shows the characteristics of the study population. When characteristics were compared between groups; no significant association was found except post-treatment levels of triglyceride ($p<0.05$*).

HMGCR and Apo E genotyping

Table 3 shows genetic analyses of non-responders and responders. Any significant associations were not found between groups ($p>0.05$).

Association of genotypes and risk factors

Some statistically significant relations were detected between genotypes and risk factors. Statistically significant associations are shown in table 4.

DISCUSSION

Statins are thought-about one in every of the foremost effective categories of medication for reducing total cholesterol and LDL-c. Even though effectiveness of treatment with statin is extremely high, still efficacy of this treatment vary essentially amongst people. It is considered that genetic background has a vital function in these variations; however involvement of single and combined mutations is not perfectly known (Poduri *et al* 2010).

In this study, we have assessed Apo E and HMGCR mutations that are concerned in the metabolism of statin and lipids with the variation in total cholesterol, triglyceride, HDL-c and LDL-c levels in statin response. Treatment with twenty mg/day of statin considerably lowers the concentrations of total cholesterol and LDL-c in plasma among fifty patients (responders). Triglyceride concentrations were conjointly reduced in responders, whereas HDL-c didn't reach statistically significant value.

In present study it was additionally found that presence of rs17244841 or rs17238540 mutations in HMGCR caused a statistically important reduction in total cholesterol and LDL-c levels. Equally Chasman *et al*. discovered that the presence of rs17244841 or rs17238540 cause a decline in total cholesterol and LDLc levels (Chasman *et al*, 2004).

Table 1: LDL cholesterol and total cholesterol levels of groups.

Parameters	Responders (n=50)	Non-responders (n=50)	p values	Responders (n=50)	Non-responders (n=50	p values	% change of responders	% change of non-responders	p values
	Baseline levels	Baseline levels		Post-treatment level	Post-treatment levels				
LDL-cholesterol	161.55± 32.99	177.52± 34.04	0.03*	86.74± 31.73	151.77± 34.25	<0.001**	46.37± 10.45	13.47± 10.83	<0.001**
Total cholesterol	243.06± 39.17	257.41± 41.02	0.06	161.27± 33.39	231.34± 44.11	<0.001**	33.69± 8.99	9.92± 9.89	<0.001**

p<0.05*, p<0.001**.

Table 2: Characteristics of the study population.

Characteristics	Groups (number of participants)		p values
	Responders (n=50)	Non-responders (n=50)	
Age (years)	59.24 ± 11.12	61.8 ± 09.53	0.21
Weight (kg)	79.72 ± 11.87	80.78 ± 10.11	0.64
Height (cm)	171.84 ± 30.05	167.26 ± 8.94	0.41
BMI (kg/m2)	29.97 ± 4.42	28.83 ± 3.21	0.89
Dyslipidemia (%)	50 (100%)	50 (100%)	1
Hypercholesterolemia (%)	50 (100%)	50 (100%)	1
Diabetes mellitus (%)	10 (20%)	10 (20%)	1
Hypertension (%)	27 (54%)	30 (60%)	0.44
Current smoking (%)	11 (22%)	13 (26%)	0.57
Alcohol consumption (%)	5 (9%)	4 (8%)	1
CVD (%)	50 (100%)	50 (100%)	1
Family history of CAD (%)	31 (62%)	25 (50%)	0.24
Triglyceride (baseline levels) (mg/dL)	161.56 ± 50.65	186.98 ± 122.49	0.13
Triglyceride (post-treatment levels) (mg/dL)	124.9 ± 37.59	170.74 ± 118.09	0.01*
HDL-c (baseline levels) (mg/dL	47.16 ± 11.87	47.24 ± 10.67	0.95
HDL-c (post-treatment levels) (mg/dL)	48.64 ± 10.44	46.06 ± 10.09	0.45
Glucose (baseline levels) (mg/dl)	117.4 ± 32.37	121.35 ± 53.31	0.62
Glucose (post-treatment levels) (mg/dL)	102.30 ± 27.17	128.10 ± 55.12	0.06

*p<0.05.

Table 3: Genetic analyses of responders and non-responders

Genes and variations	Wild type	Responders (n=50)		Wild type	Non-responders (n=50)		p values
		Homozygous mutation	Heterozygous mutation		Homozygous mutation	Heterozygous mutation	
HMGCR							
rs17244841	47	0	3	49	0	1	0.36
rs17238540	46	0	4	49	0	1	0.23
ApoE							
ε2	48	2	0	49	1	0	1
ε3	11	39	0	13	37	0	0.67
ε4	43	7	0	40	10	0	0.42

Opposite to those observations Poduri *et al*. noticed that rs17238540 mutation was considerably and severally related to a deficient response to lipid-lowering medicine in terms of LDL-C concentrations reduction (Poduri *et al* 2010). Equally Polisecki et al. found no association with the presence of rs17238540 mutation within the HMGCR gene locus and lipid`s baseline concentrations, baseline cardiovascular disease, LDL-C lowering response to statin or unproved coronary heart disease or cardiovascular disorders (Polisecki *et al* 2008).

The phenomenon has not yet been concluded which relate the HMGCR haplotypes with LDL-c levels or response of LDL-c to statin treatment. As a result LDL-c reduction is mediated by statin due to inhibition of HMGCR activity as statin competitively binds to accelerator. This genetic

Table 4: Statistically significant relations between genotypes and risk factors

Genes, variations and risk factors	Wild type	Homozygous mutation	Heterozygous mutation	p values
HMGCR (rs17244841)				
Total cholesterol (post-treatment levels)	198.82 ± 49.98	-	140.6 ± 42.68	0.016*
LDL-c (post-treatment levels)	119.58 ± 46.08	-	73.81 ± 31.89	0.011*
HMGCR (rs17238540)				
Total cholesterol (baseline levels)	252.91 ± 41.86	-	214.19 ± 25.06	0.035*
Total cholesterol (post-treatment levels)	201.29 ± 54.07	-	147.37 ± 38.67	0.013*
LDL-c (baseline levels)	171.71 ± 34.35	-	143.83 ± 26.74	0.044*
LDL-c (post-treatment levels)	119.50 ± 48.19	-	74 ± 30.59	0.008*
ApoE (ε2)				
Triglyceride (baseline levels)	161.29 ± 70.69	349.67 ± 278.90	-	0.031*
Triglyceride (post-treatment levels)	140.58 ± 74.59	274.43 ± 228.39	-	0.043*

*p< 0.05.

variation is identically associated with each baseline LDL-c concentrations and LDL-c response. This association of genetic variations might result when active site of HMGCR is structurally modified altering binding affinity for each statin and HMG-CoA. However all of the concerned SNPs are intronic, they will accelerate modifications in protein sequence by affecting template RNA splice (Krauss *et al*, 2008).

Apo E is another candidate gene to understand the regulation of cholesterol level in our body. Various studies have shown that Apo E ε4 and ε2 alleles go along with higher and lower levels of LDL-c, total cholesterol, and Apo B respectively as compared to the ε3 allele (Lehtimaki *et al* 1991, Ilveskoski *et al* 2000).It has been reported through various studies that statin response in ε4 carriers is less for reducing LDL-c and total cholesterol levels with respect to ε3 carriers. Whereas statin response in ε2 carriers have resulted in large reduction of total cholesterol and LDL-c levels in comparison to ε3 carriers during statin therapy. (Postmus *et al*, 2012, Thompson *et al,* 2005, Schmitz *et al* 2006). The occurance of such results is due to defective ε2, whereas there is high binding capability for receptor in ε4 variant in comparison to the ε3 variant (Utermann *et al*, 1987).

The rates of lipoprotein clearance are confirmed by these variations; lipoproteins are taken up with higher affinity in ε4 as compared to common ε3 variants, whereas clearance of lipoproteins is highly effective in ε3 than in ε2 variant. An ε4 variant in which the clearance of lipoprotein is done by the liver ends up in a rise in hepatic cholesterol and the hepatocyte LDL receptors were down regulaized, thus it is responsible to increase serum LDL-c, total cholesterol and apo B levels that is related to a high risk of coronary artery disease and atherosclerosis (Utermann 1987, Mahley *et al*, 1988 Liberopoulos *et al*, 2004).

The opposite condition is noticed with the ε2 variant. ε2 having lipoproteins show attenuated plasma clearance leading to upregulation of HMG-CoA synthesis. Thus, statins could also be less effective in lowering cholesterol levels in ε4 carriers, as they have already got low 5-hydroxy-3-methylglutaryl-coenzyme A reductase levels. Contrary to those findings, patients with the ε2 genotype might particularly exploit benifit from statin therapy (Schmitz *et al*, 2006). Still, it has been found by various studies that there is no significant association between ApoE SNPs and lipid levels through the statin therapy (Nieminen *et al,* 2008, Pena *et al*, 2002, Sanllehy *et al*, 1998). What is more, a meta-analysis failed to ensure the relation between Apo E mutations and lipid response statin therapy therapy (Zintzaras *et al*, 2009).

Likewise in our study no effective relation was found between Apo E genotypes, LDL-c and total cholesterol levels but statistically significant relation was found between ε2 genotype and triglyceride levels. Triglyceride levels were observed high in people who had ε2 genotype. Futhermore, Christidis *et al*. discovered that, when statin therapy, ε2 carriers had increased levels of triglyceride followed by ε4 and ε3 carriers (Christidis *et al*., 2006).Although these studies add to the growing body of literature associating Apo E variation to statin response, they are doing not to facilitate in providing a definitive description of this association (Mangravite *et al*, 2007).

CONCLUSIONS

HMGCR mutations were principally found in responders and ε4 variant of ApoE was principally found in non-responders. It was found that the presence of HMGCR mutations causes a big reduction in total cholesterol and LDL-c levels. Conjointly, the presence of ε2 variant of Apo E causes a statistically vital increase in triglyceride levels.

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