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Synergistic Effect Between Lipoprotein Lipase and Apolipoprotein C3 Genes in Determining the Severity of Coronary Artery Disease

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Abstract A number of genetic variants have been identified in the lipoprotein lipase (LPL) gene. We aimed to investigate the possible associations between *LPL* gene and apolipoprotein C3 (*APOC3*) gene polymorphisms with coronary artery disease (CAD) and its severity, as well as the interaction between these polymorphisms and classical risk factors. The *Hin*dIII variant of LPL and APOC3 were genotyped in 156 CAD patients and 154 subjects as a control group. We found that the odds ratio (OR) estimating the effect of joint exposure to H2H2 genotype of LPL and S2S2 genotype of APOC3 was significantly higher than the OR estimating the effect of each factor in the absence of the other. The present study points to a synergistic interaction between H2H2 genotype of *LPL* gene and S2S2 genotype of *APOC3* gene that leads to increased severity of CAD. Smoking, low HDL, and diabetes increased the severity of CAD in patients carrying these risky genotypes.

Keywords Lipoprotein lipase · Apolipoprotein C3 · Polymorphism · Coronary artery disease

Introduction

Coronary artery disease (CAD) has a multifactorial basis involving a number of genes and risk factors interacting to determine the development of the disease. Atherosclerosis is responsible for most cases of CAD; one of the major predisposing factors to atherosclerosis is an abnormal lipoprotein metabolism, and it may be present in over 70 % of patients with premature CAD [1]. In addition, high triglycerides (TG) and/or low HDL cholesterol (HDLc) is a common lipid phenotype which has a major role in the susceptibility to atherosclerosis [1]. Therefore, association of common gene variants in candidate genes with changes in TG and HDLc levels might be important determinants of CAD risk; the lipoprotein lipase (*LPL*) gene represents one such genes [2].

LPL is mainly synthesized by adipose tissue and muscle. It is transported and anchored to the luminal surface of the capillary endothelium by heparin sulfate proteoglycans. It is the major enzyme responsible for the hydrolysis of circulating TG-rich lipoproteins [1] and has both pro- and anti-atherogenic roles [3]. Namely, diminishing TGs in the blood can limit reduction of HDL-cholesterol mediated by cholesteryl ester transfer protein [4]. In the vessel wall, LPL activity may be associated with lipoprotein retention because of foam cell formation. LPL possibly induces cholesteryl ester accretion in smooth muscle cells during atherogenesis because of its transferase activity [5]. The anti-atherogenic role is mostly due to plasma LPL, whereas the pro-atherogenic role is mediated by LPL from vessel wall epithelial cells and macrophages [3].

The gene encoding LPL is located on chromosome 8p22, spans close to 30 kb, and contains ten exons [6]. Genetic variants can lead to lipid disorders and increase atherosclerosis risk. The *Hin*dIII polymorphism (rs320) is a base transition of thymine to guanine at position +495 in intron 8, which abolishes the restriction site for the enzyme *Hin*dIII [7].

It has been proposed that the apolipoprotein composition of lipoproteins is more closely linked to CAD than the conventional lipoprotein measurements of lipid content and density [8]. Another candidate gene that plays an important role in the metabolism of TG is the *APOC3*, which

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codes for apolipoprotein C-III (ApoC-III). ApoC-III is a major component of TG-rich lipoproteins (chylomicrons and VLDL) and a minor component of HDL, and it has been shown in vitro that ApoC-III is an inhibitor of LPL [9]. The *APOC3* gene is localized on 11q23.3. The cytosine to guanosine substitution in 3′-untranslated region of the *APOC3* gene gives rise to an *Sst*I restriction site (rs5128) and has been reported to be associated with TG levels in a number of studies [10] but not in others [11].

The exact mechanism of action of these polymorphisms is however controversial, it is probable that the effects are through lipid metabolism, although some research shows an effect independent of blood lipid levels. Among the environmental factors, tobacco smoking has been largely related to lipid metabolism. Freeman et al. [12] reported that smokers have lower HDLc and higher TG concentrations than nonsmokers. In addition, a reduced LPL activity was noticed in smokers [12].

We aim to investigate the possible associations between *LPL* gene and apolipoprotein C3 (*APOC3*) gene polymorphisms with CAD and its severity in male Egyptians, as well as the interaction between these polymorphisms and classical risk factors.

Subjects and Methods

Subjects

Study subjects were recruited among patients who underwent coronary angiography in the Cardiovascular Center at Zagazig University, for detection of the presence and extent of stenosis in coronary artery vessels according to a designed protocol. Participants with hepatic or renal disease, hormonal replacement therapy or thyroidal medication, uncontrolled diabetes, acute or systemic disease, and acute myocardial infarction within the last 3 months were excluded from the study.

The present study comprised 310 male Egyptian participants. The CAD included 156 patients with a coronary score defined as the number of coronary arteries with more than 70 % stenosis [13, 14] and subdivided into three groups: single- (SVD), two- (2VD), and three-vessel diseases (3VD). One hundred fifty-four age-matched and had the same smoking status, healthy volunteers, as controls. They were randomly recruited, had neither clinical symptoms nor electrocardiographic changes indicative of CAD, and they had no family history of CAD or stroke, therefore did not undergo coronary angiography. No study participant had taken any lipid-lowering drug. The study was approved by the ethical committee of the Faculty of Medicine, Zagazig University, and written informed consent was obtained from all participants.

Biochemical Measurements

Analyses of Lipid

Blood samples were drawn from all subjects after an overnight fast. Sera were separated immediately and stored at −20 °C. Total cholesterol and TG were measured by routine enzymatic methods (Spinreact). HDLc was determined after precipitation of the ApoB-containing lipoproteins. LDLc was calculated using the Friedewald formula [15].

Isolation of DNA

Genomic DNA was extracted from EDTA whole blood using a spin column method according to the protocol (QIAamp Blood Kit; Qiagen GmbH, Hilden, Germany).

Amplification of HindIII Polymorphism of LPL Gene

The subjects were genotyped for *Hin*dIII polymorphism of *LPL* gene by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) as described previously [16]. The region surrounding the polymorphism was amplified with following forward primers 5′-GATGTCTACCTGGATAATCAAAG-3′ and reverse primer 5′-CTTCAGCTAGACATTGCTAGTGT-3′. PCR was performed at 94 °C for 5 min followed by 30 cycles of 94 °C for 60 s, 55 °C for 60 s, and 72 °C for 60 s, then 20 cycles of 95 °C for 20 s, 56 °C for 50 s, and 72 °C for 50 s, this was followed by 7 min of extension at 72 °C. Digestion of 365 bp amplified products with *Hin*dIII yielded 365 bp for H1 allele and 205 and 160 bp fragments for H2 allele.

Amplification of APOC3-SstI Polymorphism

The polymorphic *Sst*I site of the *APOC3* gene was analyzed by PCR-RFLP method described by Singh et al. [17]. The region surrounding the polymorphism was amplified with following forward primers 5′-CATGGTTGCCTACAGGAGTTC-3′ and reverse primer 5′-TGTCGAAACACGCCTTCCAGT-3′. PCR was performed at 95 °C for 5 min followed by 35 cycles of 95 °C for 1 min, 58 °C for 45 s, 72 °C for 1 min. A final extension step at 72 °C for 7 min. Subsequently, it was subjected to digestion with *Sst*I enzyme, which cleaved the S1 allele into two fragments of 225 and 317 bp fragments.

Statistical Analysis

The results for quantitative variables were expressed as means±SD. The means of the three-genotype groups were compared in a one-way analysis of variance. Qualitative data were compared by Chi-square test; when we have one

or more cells with expected values less than 5, we used Fischer's exact test. Genotype frequencies in cases and controls were tested for Hardy–Weinberg equilibrium, and any deviation between the observed and expected frequencies was tested for significance using the chi-square test. In addition, the odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated as a measure of the association of the *Hin*dIII genotype of *LPL* gene and APOC3 genotype with severity of CAD. Multiple regression analysis was performed. To analyze possible synergism between *Hin*dIII and APOC3 genotypes in developing severe CAD we used a 4×2 table approach to calculate ORs respective 95 % CIs and *P* values, as well as synergy index (SI). It was assumed that unexposed individuals without the susceptibility genotype have a certain background risk for disease (OR_{00} is assumed to be 1); OR_{10} refers to the relative risk for disease among people without the susceptibility genotype for disease but exposed to the environmental risk factor relative to those with neither the susceptibility genotype nor exposure; OR_{01} refers to the relative risk among people with the susceptibility genotype who are not exposed to the risk factor relative to those with neither the susceptibility genotype nor exposure; OR_{11} is the ratio of disease risk among exposed people with susceptibility genotype to diseased risk among unexposed people without the susceptibility genotype. These ORs were then used in the calculation of SI= $(OR_{11}-1)/(OR_{10}+OR_{01}-2)$ [18]. A difference was considered significant at $P<0.05$. All statistical analyses were performed using SPSS.

Results

Clinical Characteristics of the Study Subjects

All the study subjects were males, and both control and patient groups were age matched and had the same smoking status (Table 1). None of the control subjects had hypertension or diabetes. Regarding the lipid profile, the levels of total cholesterol, TG, and LDLc were significantly increased in CAD patients compared with the control group. Furthermore, levels of HDLc were significantly decreased in CAD patients compared with the control group. The genotype frequencies of the LPL and APOC3 were in agreement with Hardy–Weinberg equilibrium in all groups. In the CAD group, the frequencies of LPL-H2H2 genotype and APOC3-S2S2 genotype were significantly increased compared with the control group ($P<0.001$).

LPL and APOC3 Polymorphisms Genotypes and Alleles Distribution and Severity of CAD

In 3VD patients, the frequencies of H2H2 genotype were significantly increased compared with those of patients of SVD and 2VD (85.7 versus 56.1 %) (Table 2). Subjects with LPL-H2H2 genotype were significantly more likely to have 3VD (OR=4.69 (95 % CI=1.75–14.57); $P<0.001$). Carriers of the H2 allele were significantly more likely to have 3VD (OR=3.9 (95 % CI=1.54–10.64); $P=0.001$).

In 3VD patients, the frequencies of S2S2 genotype were significantly increased compared with those of patients of SVD and 2VD (28.6 versus 6.1 %). Subjects with APOC3-S2S2 genotype were significantly more likely to have 3VD (OR=5.8 (95 % CI=1.81–19.5); $P<0.001$). Carriers of the S2 allele were significantly more likely to have 3VD (OR=2.2 (95 % CI=1.3–3.8); $P=0.003$).

Table 1 Clinical characteristics, lipid parameters, and genotypes of LPL and ApoC3 of the studied groups

Parameter	Controls	CAD	*P* value
N	154	156	
Age (years)	50.7±9.4	51.5±7.9	>0.05
Hypertension (*n* (%))	0	102 (65.4 %)	
Diabetes (*n* (%))	0	48 (30.8 %)	
Smoker (*n* (%))	98 (63.6 %)	96 (61.5 %)	>0.05
Body mass index (kg/m^2)	25.7±2	26.2 ± 2.9	>0.05
Cholesterol (mg/dl)	184.5±9.5	242.6±23.6	<0.001
Triglycerides (mg/dl)	130.7±14.2	177 ±14.3	<0.001
HDLc (mg/dl)	54.4±3.1	42.1±4	<0.001
LDLc (mg/dl)	106.8±7.2	165.1±23.1	<0.001
*Hin*dIII genotypes			
H1H1	22 (14.3)	3 (1.9)	0.004
H1H2	54 (35.1)	53 (34)	
H2H2	78 (50.6)	100 (64.1)	
APOC3 genotypes			
S1S1	140 (90.9)	84 (53.8)	<0.001
S1S2	13 (8.4)	53 (34)	
S2S2	1 (0.7)	19 (12.2)	

Synergistic Effect of LPL, APOC3 Polymorphisms and Severity of CAD

The OR estimating the effect of joint exposure to H2H2 genotype of LPL and S2S2 genotype of APOC3 was significantly higher than the OR estimating the effect of each factor in the absence of the other (OR=35.3 (95 % CI=6.7–185.1); $P<0.001$) (Table 3). Synergy index (SI=1.65).

Multiple Regression Analysis of the Severity of CAD

The presence of severity of CAD was tested for independence from other variables by multiple regression analysis (Table 4). The model included age, hypertension, diabetes, smoking, total cholesterol, TG, LDLc, HDLc, *Hin*dIII, and APOC3 polymorphisms. We found age, smoking, total

Table 2 LPL and APOC3 polymorphisms genotypes and alleles distribution and severity of CAD

	3VD (*N*=42)		1+2VD (*N*=114)		OR (95 % CI)	*P* value
	N	%	*N*	%		
*Hin*dIII genotypes						
H1H1+H1H2	0+6	14.3	3+47	43.9	1	
H2H2	36	85.7	64	56.1	4.69 (1.75–14.57)	<0.001
H2 allele	78	92.8	175	76.8	3.9 (1.54–10.64)	0.001
APOC3 genotypes						
S1S1	19	45.2	65	57.1	1	
S1S2	11	26.2	42	36.8	0.9 (0.36–2.23)	0.7
S2S2	12	28.6	7	6.1	5.8 (1.81–19.5)	<0.001
S2 allele	35	41.7	56	24.6	2.2 (1.3–3.8)	0.003

cholesterol, TG, and LDLc and low HDLc, LPL H2H2 genotype, and APOC3 S2S2 genotype to be independently related to severity of CAD.

Interaction Between LPL and APOC3 Polymorphisms with Classical Risk Factors and Its Effect in Developing Severe CAD

Hypertension did not increase the risk of 3VD in CAD patients carrying H2H2 genotype (Table 5). However, the presence of diabetes, smoking, and low HDLc increased the risk to develop 3VD in CAD patients carrying the same genotype (OR=1.8, 2.9, and 2.5, respectively; P=0.018, <0.001, and 0.002, respectively).

The presence of hypertension, diabetes, smoking and low HDLc increased the risk to develop 3VD in CAD patients carrying the S2S2 genotype of APOC3 (OR=5.7, 2.7, 17.4, and 17.4, respectively; P<0.001, 0.007, <0.001, and <0.001, respectively).

Discussion

The novel finding in the present study was the presence of a synergism between H2H2 genotype of *LPL* gene and S2S2 genotype of *APOC3* gene and the risk of developing of severe CAD in Egyptian males. To the best of our knowledge, this is the first study to report such an association.

The present study found that the risk of severe CAD was increased 7.1-fold in subjects carrying H2H2 genotype of *LPL* gene and 15.7-fold in subjects carrying S2S2 genotype of *APOC3* gene. The risk provided by H2H2 genotype of LPL was found to be positively reinforced by S2S2 genotype of APOC3 (SI=1.65). Taken together, these data suggest that individuals who are carrying both H2H2 genotype of LPL and S2S2 genotype of APOC3 are at an increased risk of suffering severe CAD. In multiple regression analysis, H2H2 genotype of LPL and S2S2 genotype of APOC3 were independent risk factors for the severity of CAD.

Previous reports are inconsistent [16, 19, 20] regarding CAD associations with these polymorphisms. Russo et al. [21] did not find any evidence of increased CAD risk associated with the S2 allele.

A growing body of evidence indicates that ApoC-III is a multifunctional protein that not only regulates the metabolism of TG-rich lipoproteins but also an important regulator of endothelial function. ApoC-III stimulates the adhesion of peripheral monocytes to endothelial cells by activating monocytes [22]. Thus, ApoC-III exerts proinflammatory effects on both monocytes and endothelial cells that are important for transendothelial migration of monocytes into the vessels' intima and development of atherosclerosis [23]. Its ability to induce endothelial dysfunction links hyperlipidemia with endothelial cell dysfunction is one mechanism by which TG-rich lipoproteins may augment inflammatory responses associated with developing atherosclerotic

Table 3 Synergistic effect of LPL, APOC3 polymorphisms, and severity of CAD

H2H2 LPL	S2S2 APOC3	1+2VD		3VD		OR (95 % CI)	*P* value	Synergy index (SI)
		N	%	*N*	%			
0	0	47	41.2	3	7.1			
0	1	3	2.6	3	7.1	15.7 (2.2–113.6)	<0.001	
1	0	60	52.6	27	64.4	7.1 (2.1–24.7)	<0.001	1.65
1	1	4	3.6	9	21.4	35.3 (6.7–185.1)	<0.001	

Table 4 Multiple regression analysis for the severity of CAD

Variable	Unstandardized coefficients		Standardized coefficients (ß)	95 % CI	*t*	*P* value
	B	Standard error				
Age	1.7E−02	0.003	0.26	0.01 to 0.02	4.9	<0.001
Hypertension	3.2E−02	0.05	0.04	−0.07 to 0.13	0.64	>0.05
Diabetes	2.9E−02	0.05	0.03	0.07 to 0.14	0.54	>0.05
Smoking	0.35	0.06	0.4	0.24 to 0.46	6.1	<0.001
Cholesterol	9.3E−03	0.001	0.5	0.007 to 0.012	7.5	<0.001
Triglycerides	4.5E−03	0.002	0.14	0.001 to 0.008	2.3	0.02
HDLc	−2.2E−02	0.006	−0.2	−0.03 to −0.009	−3.5	0.001
LDLc	8.02E−03	0.001	0.4	0.006 to 0.01	6.4	<0.001
H2H2 genotype	0.17	0.05	0.18	0.06 to 0.27	3.3	0.001
S2S2 genotype	0.18	0.08	0.15	0.023 to 0.34	2.3	0.025

lesions, thereby increasing the risk for atherosclerotic cardiovascular disease. Targeting ApoC-III could be an important new therapeutic approach to reduce coronary heart disease risk in patients with dyslipidemia [23].

Interaction Between the Classical Risk Factors and LPL and APOC3 Polymorphisms and Their Effect on Developing Severe CAD

Regarding severity of CAD, we found that the association of risk genotypes (H2H2 and S2S2) with diabetes, smoking, or low HDLc increased severity of CAD. In addition, the association of hypertension with S2S2 genotype increased the risk of severe CAD.

In a previous study [24], we demonstrated that H2H2 or S2S2 genotypes are associated with dyslipidemia and increased risk of myocardial infarction. In addition, the presence of diabetes with these polymorphisms led to an unfavorable lipid profile.

LPL is believed to be organized in an N-domain, which is important for the catalytic function of the enzyme, and a C-domain, which is important for the LPL-mediated uptake of lipoproteins by receptors on the cell surface [6]. Substitutions of amino acid which located in the N-domain may reduce enzyme activity and consequently increase TG levels as in *Hin*dIII polymorphism. As LPL activity is known to affect HDLc through its hydrolysis of TG-rich lipoproteins [25]. Reduced HDLc may result in reduced reverse cholesterol transport, indirectly promoting atherosclerosis [26].

Conclusions

The present study points to a synergistic interaction between H2H2 genotype of *LPL* gene and S2S2 genotype of *APOC3* gene that leads to increased severity of CAD. Smoking, low HDLc, and diabetes increased the severity of CAD in patients carrying these risky genotypes.

Study Limitation

Our sample size may be considered relatively small. Furthermore, the generalizability of our results is limited given the lack of ethnic, racial diversity because all patients and controls were Egyptian males. Also, it would be possible to

Table 5 Interaction between LPL and APOC3 polymorphisms with classical risk factors and its effect in developing severe CAD

Classical risk factors	H2H2 of LPL		S2S2 of APOC3	
	OR (95 % CI)	*P* value	OR (95 % CI)	*P* value
Without hypertension	0.6 (0.2–1.1)	>0.05	2.1 (1.6–2.7)	<0.001
With hypertension	0.9 (0.6–1.7)	>0.05	5.7 (2.8–11.6)	<0.001
Without diabetes	0.9 (0.7–1.2)	>0.05	0.9 (0.6–1.4)	>0.05
With diabetes	1.8 (1.1–3)	0.018	2.7 (1.3–5.9)	0.007
Without smoking	0.7 (0.6–1.04)	>0.05	2.2 (1.6–2.9)	<0.001
With smoking	2.9 (1.7–5.2)	<0.001	17.4 (4.5–67.6)	<0.001
Without low HDLc	1.3 (1.1–1.6)	0.012	0.8 (0.5–1.3)	>0.05
With low HDLc	2.5 (1.3–4.7)	0.002	17.4 (4.5–67.6)	<0.001

compare the disease group to a group of patients that had been catheterized (for cause) but were not shown to have CAD. This group of patients may serve as a more appropriate control group.

Conflict of Interest None

References

1. Bainton, D., Miller, N. E., Bolton, C. H., Yarnell, J. W., Sweetnam, P. M., Baker, I. A., et al. (1992). Plasma triglycerides and high-density lipoprotein cholesterol as predictors of ischemic heart disease in British men: the Caerphill and Speedwell Collaborative Heart Disease Studies. *British Heart Journal, 68*, 60–66.
2. Wittrup, H. H., Tybjaerg-Hansen, A., & Nordestgaard, B. G. (1999). Lipoprotein lipase mutations, plasma lipids and lipoproteins, and risk of ischemic heart disease: a meta-analysis. *Circulation, 99*, 2901–2907.
3. Goldberg, I. J. (1996). Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *Journal of Lipid Research, 37*, 693–707.
4. Newnham, H. H., & Barter, P. J. (1992). Changes in particle size of high density lipoproteins during incubation with very low density lipoproteins, cholesteryl ester transfer protein and lipoprotein lipase. *Biochimica et Biophysica Acta, 1125*, 297–304.
5. Clee, S. M., Bissada, N., Miao, F., Miao, L., Marais, A. D., Henderson, H. E., et al. (2000). Plasma and vessel wall lipoprotein lipase have different roles in atherosclerosis. *Journal of Lipid Research, 41*, 521–531.
6. Murthy, V., Julien, P., & Gagne, C. (1996). Molecular pathobiology of the human lipoprotein lipase gene. *Pharmacology and Therapeutics, 70*, 101–135.
7. Gotoda, T., Yamada, N., Murase, T., Shimano, H., Shimada, M., Harada, K., et al. (1992). Detection of three separate DNA polymorphisms in the human lipoprotein lipase gene by gene amplification and restriction endonuclease digestion. *Journal of Lipid Research, 33*, 1067–1072.
8. Alaupovic, P. (1996). Significance of apolipoproteins for structure, function, and classification of plasma lipoproteins. *Methods in Enzymology, 263*, 32–60.
9. McConathy, W. J., Gesquiere, J. C., Bass, H., Tartar, A., Fruchart, J. C., & Wang, C. S. (1992). Inhibition of lipoprotein lipase activity by synthetic peptides of apolipoprotein C-III. *Journal of Lipid Research, 33*, 995–1003.
10. Hoffer, M. J. V., Sijbrands, E. J. G., De Man, F. H. A. F., Havekes, L. M., & Smelt, A. H. M. (1998). Increased risk of endogenous hypertriglyceridaemia is associated with an apolipoprotein C3 haplotype specified by the *Sst*I polymorphism. *European Journal of Clinical Investigation, 28*, 807–812.
11. Kee, F., Amouyel, P., Fumeron, F., Arveiler, D., Cambou, J. P., Evans, A., et al. (1999). Lack of association between genetic variations of apo A-I-CIII-A-IV gene cluster and myocardial infarction in a sample of European male: ECTIM study. *Atherosclerosis, 145*, 187–195.
12. Freeman, D. J., Caslake, M. J., Griffin, B. A., Hinnie, J., Tan, C. E., Watson, T. D. G., et al. (1998). The effect of smoking on post-heparin lipoprotein and hepatic lipase, cholesteryl ester transfer protein and lecithin: cholesterol acyl transferase activities in human plasma. *European Journal of Clinical Investigation, 28*, 584–591.
13. Walk, R., Berger, P., Lennon, R. J., Brilakis, E. S., Johnson, B. D., & Somers, V. K. (2004). Plasma leptin and prognosis in patients with established coronary atherosclerosis. *Journal of the American College of Cardiology, 44*, 1819–1824.
14. Vlietstra, R. E., Kronmal, R. A., Frye, R. L., Seth, A. K., Tristani, F. E., & Killip, T., 3rd. (1982). Factors affecting the extent and severity of coronary artery disease in patients enrolled in the coronary artery surgery study. *Arteriosclerosis, 2*, 208–15.
15. Frieldewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry, 18*, 499–502.
16. Mattu, R. K., Needham, E. W. A., Morgan, R., Rees, A., Hackshaw, A. K., Stocks, J., et al. (1994). DNA variants at the *LPL* gene locus associate with angiographically defined severity of atherosclerosis and serum lipoprotein levels in a Welsh population. *Arteriosclerosis and Thrombosis, 14*, 1090–1097.
17. Singh, P., Singh, M., Bhatnagar, D. P., Kaur, T., & Mastana, S. (2008). Apolipoprotein C3 (*Sst*I) gene variability in Northwest India: a global perspective. *International Journal of Human Genetics, 8*, 51–60.
18. Rothman, K. J. (1974). Synergy and antagonism in cause–effect relationship. *American Journal of Epidemiology, 99*, 385–388.
19. Pasalic, D., Ferencak, G., Grskovic, B., Sesto, M., & StavljeniC-Rukavina, A. (2006). Association of two genetic variations of lipoprotein lipase, S447X and *Hin*dIII, with coronary artery disease and hypertriglyceridemia. *Collegium Antropologicum, 30*, 549–554.
20. Ordovas, J. M., Civeira, F., Genest, J., Jr., Craig, S., Robbins, A. H., Meade, T., et al. (1991). Restriction fragment length polymorphisms of the apolipoprotein A-I, C-III, A-IV gene locus. Relationship with lipids, apolipoproteins, and premature coronary artery disease. *Atherosclerosis, 87*, 75–86.
21. Russo, G. T., Meigs, J. B., Cupples, L. A., Demissie, S., Otvos, J. D., Wilson, P. W. F., et al. (2001). Association of the Sst-I polymorphism at the *APOC3* gene locus with variations in lipid levels, lipoprotein subclass profiles and coronary heart disease risk: the Framingham offspring study. *Atherosclerosis, 158*, 173–181.
22. Kawakami, A., Masanori, A., Libby, P., Alcaide, P., Luscinskas, F. W., & Sacks, F. M. (2006). Apolipoprotein CIII in apolipoprotein B lipoproteins enhances the adhesion of human monocytic cells to endothelial cells. *Circulation, 113*, 691–700.
23. Bobik, A. (2008). Apolipoprotein CIII and atherosclerosis beyond effects on lipid metabolism. *Circulation, 118*, 702–704.
24. Abd El-Aziz, T. A., Mohamed, R. H., & Hashem, R. M. (2011). Association of lipoprotein lipase and apolipoprotein C-III genes polymorphism with acute myocardial infarction in diabetic patients. *Molecular and Cellular Biochemistry, 354*, 141–150.
25. Magill, P., Rao, S. N., Miller, N. E., Nicoll, A., Brunzell, J., Hilaire, J. S., et al. (1982). Relationships between the metabolism of high density and very low density lipoproteins in man: studies of apolipoprotein kinetics and adipose tissue lipoprotein lipase activity. *European Journal of Clinical Investigation, 12*, 113–120.
26. Araújo, L. M. Q., Cendoroglo, M. S., Gigek, C. O., Chen, E. S., & Smith, M. A. C. (2010). Association of lipase lipoprotein polymorphisms with high-density lipoprotein and triglycerides in elderly men. *Genetics and Molecular Research, 9*(1), 89–96.