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ORIGINAL ARTICLE

Cholesterol-related gene variants are associated with diabetes in coronary artery disease patients

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Abstract

Coronary artery disease (CAD) which is a complex cardiovascular disease is the leading cause of death worldwide. The changing prevalence of the disease in different ethnic groups pointing out the genetic background of CAD. In this study, we aimed to evaluate the contribution of selected cholesterol metabolism-related gene polymorphisms to CAD presence. A total of 493 individuals who underwent coronary angiography were divided into 2 groups: normal coronary arteries (≤ 30% stenosis) and critical disease (≥ 50% stenosis). Individuals were genotyped for *APOC1* (rs11568822), *APOD* (rs1568565), *LIPA* (rs13500), *SORL1* (rs2282649), and *LDLR* (rs5930) polymorphisms using hydrolysis probes in Real-Time PCR. Blood samples were drawn before coronary angiography and biochemical analyses were done. The results were statistically evaluated. When the study group was stratified according to CAD, the minor allele of *APOD* polymorphism was found related to decreased risk for T2DM in the non-CAD group. In logistic regression analysis adjusted for several confounders, *LDLR* rs5930 polymorphism was found associated with T2DM presence in the male CAD group [OR = 0.502, 95%CI (0.259–0.974), $p = 0.042$]. Besides, *APOD* and *LIPA* polymorphisms were shown to affect serum lipid levels in non-CAD T2DM patients ($p < 0.05$). The minor allele of *APOC1* was found associated with triglyceride levels in males independent of CAD status. Besides, *LDLR* minor allele carrier females had elevated HbA1c and glucose levels independent from CAD status in the whole group. The cholesterol metabolism-related gene polymorphisms were found associated with T2DM and biochemical parameters stratified to sex, CAD, and T2DM status.

Keywords Polymorphism · Coronary artery disease · Diabetes mellitus · Cholesterol

Introduction

Coronary artery disease (CAD) which is a complex cardiovascular disease is the leading cause of death worldwide despite all measures [1]. Although risk factors such as smoking, diabetes, hypertension, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) are identified, the changing prevalence of the disease at different ethnic groups pointing out the genetic background of CAD [2, 3]. In this context, the importance of identifying new candidate genes, and thus developing novel therapeutic approaches for atherosclerosis can be overemphasized. Since atherosclerosis is the major cause of CAD, lipid metabolism plays a central role in it, and genes related to cholesterol metabolism expected to be involved in the development of CAD [3].

Apolipoprotein D (ApoD) is mainly a component of HDL but also presents in very-low-density lipoproteins (VLDL) and LDL [4]. ApoD protein is widely expressed, unlike other apolipoproteins which are generally expressed in the liver [5]. Initial studies on ApoD showed that ApoD interacts with various ligands, including cholesterol [5]. Besides, previous reports were identified that the *APOD* gene polymorphisms are related to the characteristic lipid profile of metabolic syndrome [6]. The gene product of *APOC1* is a constituent of chylomicrons, VLDL, and HDL and is involved in the regulation of the exchange of cholesterol between lipoproteins and removal of cholesterol from tissues [7, 8]. The

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low-density lipoprotein receptor (LDLR) is a cell surface protein that binds to the LDL which are the primary carriers of the cholesterol in the blood [9]. LDLR binds to the apoB particle on the surface of LDL and the receptor-ligand complex internalized by endocytosis [9]. Previous studies showed that the upregulation of LDLR protein decreases the risk of cardiovascular events by reducing the plasma LDL-C levels [10, 11]. Sortilin Related Receptor 1 (SORL1) which is also known as LDLR Relative with 11 Ligand-Binding Repeats (LR11) is a member of the LDLR family and overexpressed in atherosclerotic plaques [12]. In previous studies, soluble LR11 levels were found correlated with intima-media thickness in carotid arteries in dyslipidemic subjects and adverse clinical outcomes in CAD patients [13, 14]. Lysosomal acid lipase (LAL) which is encoded by the *LIPA* gene is the key lysosomal enzyme for hydrolyzing cholesteryl ester and triglycerides into free cholesterol [15]. In previous animal studies, when *LDLR* deficient mice are treated with targeted delivery of LAL to macrophages, the progression of the atherosclerotic lesion was prevented, and early lesions were eliminated [16].

In this study, it was aimed to determine the genetic association of coronary artery disease with cholesterol metabolism gene polymorphisms. For this aim *APOC1* (rs11568822), *APOD* (rs1568565), *LIPA* (rs13500), *SORL1*(rs2282649), and *LDLR* (rs5930) gene polymorphisms were selected and the effects of these polymorphisms on CAD development and known metabolic risk factors such as plasma lipid levels and type 2 diabetes mellitus (T2DM) were investigated in Turkish CAD patients.

Material and methods

Study design and subjects

The study samples were recruited from Ufuk University Cardiology Department between 2014 and 2017; all samples were aged 18 years or over and underwent invasive coronary angiography due to stable angina pectoris, ischemia, and acute coronary syndrome. Selected 493 Turkish individuals [276 patients > 50% luminal stenosis narrowing and 217 non-CAD controls (< 30% stenosis)] were enrolled in the study (Supp. Table S1). Two independent operators were evaluated coronary luminal narrowing following the guidelines of the American College of Cardiology/American Heart Association (ACC/AHA) for the classification of coronary lesions. Patients with a history of previous coronary bypass graft surgery, ongoing decompensated (New York Heart Association Functional Class IV) heart failure, advanced hepatic or renal failure, a life expectancy of less than a year, and known malignancy (except basal cell carcinoma and squamous cell carcinoma of the skin in full remission), and those with an active infectious or inflammatory disease or a rheumatological disease were excluded from the study. Patients with a previous history of coronary brachytherapy and previous exposure to cholesteryl-ester transfer protein (CETP) inhibitors or PCSK9 inhibitors were also excluded from the study. SYNTAX (Synergy between percutaneous coronary intervention with Taxus and Cardiac Surgery) Scores and Gensini Scores were calculated for each sample following angiography to evaluate the complexity and severity of CAD. SYNTAX score which is used for the assessment of the complexity of CAD was calculated using an online calculator (version 2.28, www.syntaxscore.com) while the Gensini score was determined according to the degree of stenosis score, multiplying factor that depends on the region of the stenosis and collateral adjustment factor [17, 18]. For further analysis, the study group was classified according to the T2DM status with CAD status and according to sex independent from CAD status. The whole sample collection and analysis processes were conducted in compliance with the ethical guidelines of the Declaration of Helsinki and approved by the Institutional Review Board at Istanbul University (Approval no: 377879). The written informed consent was obtained from all participants and all experiments were performed following the approved guidelines and regulations.

Laboratory test

Blood serum samples were collected before coronary angiography and stored at − 80 °C until analyzed. Any lipemic, icterus, and hemolysis specimens were excluded. Analyses of biochemical parameters were performed. Concentrations of total cholesterol (TC), TG, fasting glucose, HDL-C, and LDL-C were measured by UniCel DxC 800 (Beckman Coulter, USA). Hemoglobin levels were analyzed using the High-Performance Liquid Chromatography (HPLC) system. The results were given as the ratio of glycosylated hemoglobin (HbA1c) to total hemoglobin in percentages.

Genetics analysis

Determination of the candidate genes genotypes

DNA was extracted from peripheral blood leucocytes using the inorganic method [19]. Selected 493 subjects (276 CAD and 217 non-CAD) were examined for *APOC1* (rs11568822), *APOD* (rs1568565), *LIPA* (rs13500), *SORL1*(rs2282649), and *LDLR* (rs5930) polymorphisms. Genotyping was performed using hydrolysis probes in Real-Time PCR LightCycler® 480 (Roche Life Science, USA). DNA amplification was set up in 96-well plates (Roche Life Science, USA) with typical 10 µl PCR

Table 1 Genotype frequencies of selected polymorphisms stratified according to CAD, T2DM status, and sex

LDLR (rs5390)	non-CAD			Male non-CAD			Female non-CAD		
	non-T2DM	T2DM	*p-value*	non-T2DM	T2DM	*p-value*	non-T2DM	T2DM	*p-value*
CC	39.9	35.9	*0.618*	33.3	39.3	*0.798*	45.0	33.3	*0.362*
CT	41.3	48.4		47.6	46.4		36.3	50.0	
TT	18.9	15.6		19.0	14.3		18.8	16.7	
CT/TT	60.1	64.1	*0.592*	66.7	60.7	*0.583*	55.0	66.7	*0.238*
C allele	60.5	60.1	*0.949*	57.1	62.5	*0.498*	63.1	58.3	*0.487*
T allele	39.5	39.9		42.9	37.5		36.9	41.7	
LDLR (rs5390)	CAD			Male CAD			Female CAD		
	non-T2DM	T2DM	*p-value*	non-T2DM	T2DM	*p-value*	*p-value*	*p-value*	*p-value*
CC	35.6	41.1	*0.647*	33.3	51.4	***0.050***	43.3	27.3	*0.089*
CT	48.9	44.2		47.6	36.5		53.3	54.4	
TT	15.6	14.7		19.0	12.2		3.3	18.2	
CT/TT	64.4	58.9	*0.355*	66.7	48.6	***0.016***	56.7	72.7	*0.132*
C allele	60.0	63.2	*0.453*	57.1	69.6	***0.017***	70.0	54.5	*0.051*
T allele	40.0	36.8		42.9	30.4		30.0	45.5	
APOD (rs1568565)	non-CAD			Male non-CAD			Female non-CAD		
	non-T2DM	T2DM	*p-value*	non-T2DM	T2DM	*p-value*	non-T2DM	T2DM	*p-value*
TT	29.3	43.8	*0.120*	28.6	53.6	*0.067*	29.9	36.1	*0.529*
TC	50.7	42.2		58.7	35.7		44.2	47.2	
CC	20.0	14.1		12.7	10.7		26.0	16.7	
TC/CC	70.7	56.3	***0.043***	71.4	46.4	***0.022***	70.1	63.9	*0.507*
T allele	54.6	64.8	*0.054*	57.9	71.4	*0.085*	51.9	59.7	*0.275*
C allele	45.4	35.2		42.1	28.6		48.1	40.3	
APOD (rs1568565)	CAD			Male CAD			Female CAD		
	non-T2DM	T2DM	*p-value*	non-T2DM	T2DM	*p-value*	non-T2DM	T2DM	*p-value*
TT	40.0	31.0	*0.312*	41.2	31.1	*0.281*	36.4	30.9	*0.352*
TC	38.5	44.2		41.2	43.2		30.3	45.5	
CC	21.5	24.8		17.6	25.7		33.3	23.6	
TC/CC	60.0	69.0	*0.127*	58.8	68.9	*0.171*	63.9	69.1	*0.598*
T allele	59.3	53.1	*0.154*	61.8	52.7	*0.089*	51.5	53.6	*0.785*
C allele	40.7	46.9		38.2	47.3		48.5	46.4	

Genotype distributions and allele frequencies are expressed in percentages. Chi-square test was used for genotype frequencies with T2DM status. Allele frequencies were determined using the allele counting method. Significant p-values ($p < 0.05$) are indicated in bold

CAD Coronary artery disease, *T2DM* Type 2 diabetes mellitus

reaction consisted of 2 µl LightCycler® 480 Genotyping Master (Roche Life Science, USA) ready mix, 0.2 µl probes, and 0.2 µl primers, 6.6 µl distilled water. 1 µl (50 ng) genomic DNA was added to the PCR mixture. PCR was carried out using the following conditions: 95 °C for 10 min; 95 °C for 10 s, 56 °C for 30 s, 72 °C 1 s (45 cycles). The end-point analysis was performed for the discrimination of the alleles.

Statistical analysis

Hardy–Weinberg equilibrium was computed to the expected genotype distribution. The genotype distributions were compared using the chi-square test. Due to skewed distribution TG and HbA1c logarithmically transformed for analyses and expressed in geometric means. The two-tailed t-test was used to compare normally distributed continuous variables and expressed as means and standard deviation (SD), while categorical variables were compared using the chi-square test. Univariate analysis of variances adjusted for several factors

was used for the determination of the association between polymorphisms and biochemical parameters and expressed in the mean and standard error of the mean (SEM). Logistic regression models were used to derive maximum odds ratio (OR) estimates and associated 95% confidence intervals (CIs), adjusted for several factors as confounders. A p-value < 0.05 was considered significant. All statistical analyses were performed using IBM SPSS (v23.0, IBM Corporation, USA).

Results

Baseline characteristics of study subjects

Baseline characteristics of CAD (mean age; 63.4 ± 10.6, 67.0% male) and non-CAD (mean age; 57.7 ± 11.7, 43.8% male) groups were compared, and results were summarized in Supp. Table 1. The frequencies of lipid-lowering and antidiabetic drug usages were higher in the CAD group compared to non-CAD ($p < 0.01$). Besides, the Gensini score, SYNTAX score, and percentage of stenosis were also higher in the CAD group when compared to non-CAD ($p < 0.01$, Supp. Table S1).

Associations of selected polymorphisms with T2DM

When the study population was grouped according to the T2DM status in addition to CAD and non-CAD classification, in the non-CAD group it was found that minor allele (C allele) carriage of *APOD* rs1568565 polymorphism is more prevalent in the non-T2DM group ($p = 0.043$, Table 1). For further analysis, the effect of *APOD* minor allele carriage status on T2DM was examined in CAD and non-CAD groups stratified according to sex. In the male non-CAD group, *APOD* minor allele carriage was observed in a higher percentage in non-T2DM ($p = 0.022$, Table 1). In a logistic regression analysis adjusted for BMI, age, and lipid-lowering drug usage, it was found that male minor allele carriers of *APOD* polymorphism had a lower risk for T2DM in the non-CAD group (OR = 0.302, [95% CI 0.111–0.821], $p = 0.019$, Table 2).

Differences in *LDLR* rs5930 polymorphism genotype distributions ($p = 0.050$, Table 1) and allele frequencies ($p = 0.017$, Table 1) were observed among non-T2DM and T2DM groups in the male CAD group. Also, male CAD carriers of the minor allele of *LDLR* polymorphism were found in a higher percentage in the non-T2DM group ($p = 0.016$, Table 1). In logistic regression analysis adjusted for BMI, age, and lipid-lowering drug usage, it was found that minor allele carriage of *LDLR* polymorphism lowers the risk for T2DM (OR = 0.502 [95% CI 0.259–0.974] $p = 0.042$, Table 2).

There were no statistically significant differences between groups in genotype distributions and allele frequencies of selected *APOC1*, *SORL1*, and *LIPA* polymorphisms in CAD and non-CAD groups in analyses stratified according to T2DM status and sex ($p > 0.05$).

Associations between biochemical parameters and selected polymorphisms in CAD and T2DM subgroups

When the lipid profiles of individuals were examined, in a univariate analysis which is adjusted to age, sex, BMI, and lipid-lowering drug usage it was observed that *APOD* rs1568565 minor allele carriers have increased TC levels in non-CAD with T2DM group ($p = 0.004$, Fig. 1). In the univariate analysis with these same confounders, minor allele carriage of selected polymorphism of *LIPA* was found associated with elevated HDL-C levels in the non-CAD T2DM group ($p = 0.031$, Fig. 1).

Genotype distributions and allele frequencies of polymorphisms in CAD and non-CAD groups

Genotype distributions of *APOC1* (rs11568822), *APOD* (rs1568565), *LIPA* (rs13500), *SORL1* (rs2282649), and *LDLR* (rs5930) polymorphisms were given in Table 3. The genotype distributions and allele frequencies did not show statistically significant differences between non-CAD and CAD groups. The distributions of genotypes were in Hardy–Weinberg equilibrium for all polymorphisms.

Associations between biochemical parameters and selected polymorphisms in CAD and non-CAD

Association between genotype and lipid profiles which includes TC, LDL-C, HDL-C, and TG levels and other known risk factors such as BMI, HbA1c, and fasting glucose levels were examined in CAD and non-CAD groups. Minor allele carriage of *APOC1* rs11568822 and *LDLR* rs5930 polymorphisms were found associated with decreased HbA1c and HDL-C levels in the non-CAD group, respectively (Supp. Table S2). In addition, an association was found between minor allele carriage of *APOC1* polymorphism and elevated TG levels in the CAD group ($p = 0.034$, Supp. Table 2). When the study population classified according to sex independent from CAD status, male minor allele carriers of *APOC1* polymorphism ha higher TG levels in a univariate analysis adjusted to age, BMI, and lipid-lowering drug usage ($p = 0.003$, Fig. 2). Besides, in a univariate analysis adjusted to age, BMI, lipid-lowering, and antidiabetic drug usage, the minor allele of *LDLR* polymorphism was found associated with higher HbA1c and glucose levels in females independent from CAD status ($p < 0.05$, Fig. 2). No

Table 2 The effects of the minor allele of the *APOD* and *LDLR* polymorphisms on T2DM stratified according to sex and CAD status

		Non-CAD		Male Non-CAD		Female Non-CAD	
		β-coefficient (95% CI)	*p-value*	β-coefficient (95% CI)	*p-value*	β-coefficient (95% CI)	*p-value*
APOD (rs1568565)	Sex (female)	1.035 (0.555–1.929)	*0.914*				
	BMI	1.086 (1.004–1.175)	***0.040***	1.058 (0.942–1.189)	*0.341*	1.136 (1.013–1.274)	***0.029***
	Age (years)	1.005 (0.978–1.032)	*0.726*	1.017 (0.978–1.058)	*0.409*	0.993 (0.955–1.034)	*0.743*
	Lipid-lowering drug use	0.838 (0.429–1.637)	*0.604*	1.358 (0.471–3.914)	*0.571*	0.607 (0.246–1.497)	*0.278*
	APOD TC + CC[a]	0.586 (0.311–1.103)	*0.098*	0.302 (0.111–0.821)	***0.019***	0.939 (0.383–2.299)	*0.890*
		CAD		Male CAD		Female CAD	
APOD (rs1568565)	Sex (female)	0.478 (0.275–0.831)	***0.009***				
	BMI	1.125 (1.053–1.201)	***0.001***	1.176 (1.075–1.286)	***0.001***	1.046 (0.940–1.163)	*0.411*
	Age (years)	1.019 (0.994–1.045)	*0.143*	1.037 (1.006–1.070)	***0.021***	0.970 (0.922–1.020)	*0.238*
	Lipid-lowering drug use	1.175 (0.692–1.993)	*0.551*	1.315 (0.679–2.547)	*0.417*	1.028 (0.400–2.640)	*0.954*
	APOD TC + CC[a]	1.567 (0.910–2.698)	*0.105*	1.775 (0.887–3.554)	*0.105*	1.404 (0.549–3.588)	*0.479*
		Non-CAD		Male Non-CAD		Female Non-CAD	
LDLR (rs5390)	Sex (female)	1.080 (0.583–1.999)	*0.807*				
	BMI	1.084 (1.004–1.172)	***0.040***	1.051 (0.939–1.177)	*0.388*	1.164 (1.035–1.309)	***0.011***
	Age (years)	1.003 (0.977–1.030)	*0.825*	1.004 (0.968–1.042)	*0.816*	0.995 (0.956–1.037)	*0.822*
	Lipid-lowering drug use	0.900 (0.461–1.757)	*0.758*	1.438 (0.502–4.117)	*0.498*	0.601 (0.240–1.505)	*0.277*
	LDLR CT + TT[b]	1.221 (0.649–2.296)	*0.536*	0.661 (0.248–1.760)	*0.407*	2.236 (0.912–5.486)	*0.079*
		CAD		Male CAD		Female CAD	
LDLR (rs5390)	Sex (female)	0.407 (0.232–0.715)	***0.002***				
	BMI	1.124 (1.053–1.200)	***0.001***	1.167 (1.069–1.275)	***0.001***	1.032 (0.923–1.155)	*0.577*
	Age (years)	1.023 (0.997–1.049)	*0.081*	1.041 (1.009–1.074)	***0.013***	0.970 (0.921–1.023)	*0.262*
	Lipid-lowering drug use	1.195 (0.702–2.035)	*0.551*	1.363 (0.700–2.652)	*0.362*	1.114 (0.416–2.985)	*0.830*
	LDLR CT + TT[b]	0.764 (0.448–1.304)	*0.324*	0.502 (0.259–0.974)	***0.042***	2.150 (0.820–5.638)	*0.120*

[a]For TC + CC genotypes, the TT genotype is considered as reference

[b]For CT + TT genotypes, the CC genotype is considered as reference. In the logistic regression analyses, 471 individuals (193 T2DM), 295 males (102 T2DM), and 201 females (91 T2DM) were examined. Sex, BMI, age, and lipid-lowering drug usage status parameters were used as confounders

Significant *p*-values ($p < 0.05$) are indicated in bold. *CAD* Coronary artery disease, *CI* Confidence interval, *T2DM* Type 2 diabetes mellitus

statistically significant associations were observed between *SORL1, LIPA,* and *APOD* polymorphisms and biochemical parameters in CAD and non-CAD groups.

Discussion

CAD is one of the most common cardiovascular diseases and is one of the leading causes of death at present [1]. Atherosclerosis is characterized by lipid accumulation, inflammatory response, cell death, and fibrosis in the arterial wall, which is the pathological basis for atherosclerotic CAD [3]. In this study, cholesterol metabolism-related gene polymorphisms were selected and studied among CAD patients and non-CAD controls. The results of the present study pointed out that *APOD* rs1568565, *LDLR* rs5930 polymorphisms are associated with T2DM status and its indicators such as HbA1c and glucose levels, while *APOC1* rs11568822 and *LIPA* rs13500 polymorphisms are related to the lipid profile.

The associations between *LDLR* gene polymorphisms and CAD presence are widely studied among different ethnic populations [20–22]. On the other hand, there is a limited number of studies investigating the *LDLR* rs5930 polymorphism and they are mostly conducted in Alzheimer's disease patients [23]. In this study, an association was found between rs5930 polymorphism and T2DM presence in a sex-specific manner. The rs5930 polymorphism has shown protective effects against T2DM in male CAD

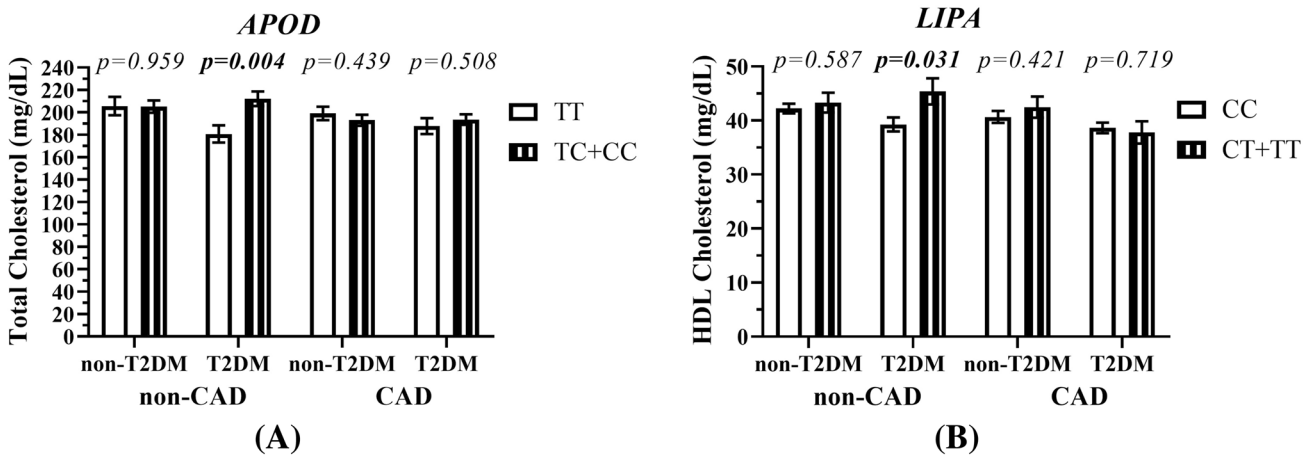

Fig. 1 Estimated mean and standard error of mean values for total cholesterol levels across the genotypes of *APOD* rs1568565 and HDL cholesterol levels across the genotypes of *LIPA* rs13500 polymorphisms were given in groups stratified according to CAD and T2DM status after adjustment for age, sex, BMI, and usage of lipid-lowering drugs. **A** Total cholesterol levels were found higher in C allele carrier T2DM patients of *APOD* rs1568565 polymorphism in the non-CAD group. **B** HDL cholesterol levels were elevated in *LIPA* rs13500 minor allele carrier T2DM patients in the non-CAD group

patients. Although there is no association between CAD, and T2DM presence and genotype distributions and allele frequencies in females, fasting glucose levels and HbA1c were found significantly higher in *LDLR* rs5930 minor allele carriers independent from CAD status. In a previous study that investigates the effect of another synonymous variant of the *LDLR* gene, rs688 polymorphism was found associated with cholesterol levels only in female individuals [24]. Zu et al. suggested that the rs688 polymorphism alters the exon splicing efficiency. Zu et al. pointed out that the association between cholesterol levels and the polymorphism is a result of alteration of the exon splicing efficancy [24]. According to RegulomeDB rs5930 polymorphism has a score of 0.61 while score 1 represents the variation is most likely regulatory [25]. It is possible that rs5930 polymorphism has a similar regulatory effect to rs688 in the region and could alter the activity of the gene. Besides, in previous reports, it was found that estradiol treatment positively regulates the expression of *LDLR* in vitro [26, 27]. Also, it was observed that postmenopausal women had lower levels of estradiol when compared to men [28]. The sex-specific result in the present study could be a result of the association between estradiol levels and *LDLR* expression. This study is the first study that shows the association between rs5930 polymorphism and its effects on T2DM status and disease-related parameters. The nature of the relation between LDLR and T2DM might be a result of the insulin receptor (IR) and LDLR complex. In previous studies, it was demonstrated that an intracellular co-association and co-localization exists for IR and LDLR in HepG2 and HUVEC cells [29, 30]. However, whether and how the rs5930 polymorphism affects the interaction of LDLR and IR remains unclear.

While the association between *APOD* rs1568565 polymorphism and CAD presence has not been previously investigated, the effects of other polymorphisms of *APOD* gene on CAD risk factors such as lipid profiles, T2DM, and insulin resistance were studied [6, 31, 32]. Also, animal studies indicated that the change of expression levels of *APOD* alters the insulin levels along with the lipid metabolism [33, 34]. In this study, the minor allele of *APOD* rs1568565 polymorphism was found associated with TC levels. In addition, the minor allele of rs1568565 polymorphism was found protective against T2DM. The mechanisms which lead to these results remain unclear and functional studies along with the studies with a larger sample size needs to be conducted for the confirmation of these associations.

The apoC-I protein is an important player in both lipid metabolism and inflammatory response. Many studies investigate the effects of gene polymorphisms in *APOC1* on the development and progression of several different diseases including CAD, polycystic ovary syndrome, and Alzheimer's disease [23, 35, 36]. Olsson et al. conducted a study in CAD patients and controls which indicates *APOC1* rs11568822 polymorphism that also known as HpaI is associated with TG levels [36]. Also, in the same study, no association was found between genotype distributions of rs11568822 and CAD presence [36]. Similar to these results, in the present study minor allele carrier male subjects showed higher serum TG levels although no association was found between genotype distributions and CAD status. The reason for the higher TG levels could be the fact that rs11568822 polymorphism of *APOC1* results

Table 3 Genotype frequencies of selected polymorphisms

	Study population % (n)	CAD % (n)	non-CAD % (n)
APOC1 Genotype frequency			
CGTTdel	75.8 (364)	76.5 (205)	75.0 (159)
ins/del	22.1 (106)	21.6 (58)	22.6 (48)
CGTTins	2.1 (10)	1.9 (5)	2.4 (5)
ins/del + CGTTins	24.2 (116)	23.5 (63)	25 (53)
CGTTdel allele	86.9	87.3	86.3
CGTTins allele	13.1	12.7	13.7
APOD Genotype frequency			
TT	34.8 (163)	35.6 (94)	33.8 (69)
TC	44.2 (207)	41.3 (109)	48.0 (98)
CC	20.9 (98)	23.1 (61)	18.1 (37)
TC + CC	65.2 (305)	64.4 (170)	66.2 (135)
T allele	56.9	56.2	57.8
C allele	43.1	43.8	42.2
LIPA Genotype frequency			
CC	79.1 (379)	78.1 (211)	80.4 (168)
CT	20.5 (98)	21.5 (58)	19.1 (40)
TT	0.4 (2)	0.4 (1)	0.5 (1)
CT + TT	20.9 (100)	21.9 (59)	19.6 (41)
C allele	89.4	88.9	90.0
T allele	10.6	11.1	10.0
LDLR Genotype frequency			
GG	38.4 (181)	38.3 (101)	38.6 (80)
GA	45.2 (213)	46.6 (123)	43.5 (90)
AA	16.3 (77)	15.2 (40)	17.9 (37)
GA + AA	61.9 (290)	61.7 (163)	61.4 (127)
G allele	61.0	61.6	60.4
A allele	39.0	38.4	39.6
SORL1 Genotype frequency			
CC	47.4 (224)	49.4 (130)	45.4 (94)
CT	41.1 (193)	41.1 (108)	41.1 (85)
TT	11.3 (53)	9.5 (25)	13.5 (28)
CT + TT	52.3 (246)	50.6 (133)	54.6 (113)
C allele	68.2	70.0	65.9
T allele	31.8	30.0	34.1

Allele frequencies were given as percentages. The frequency of alleles was computed using the gene-counting method. Chi-square test was used for genotype frequencies with CAD status. No statistically significant differences between CAD and non-CAD groups in genotype distributions and allele frequencies were observed ($p > 0.05$)

in increased expression of the gene [37]. In a previous study conducted in the apoe$^{-/-}$ mice it has been demonstrated that *APOC1* overexpression causes increased serum TG levels as a result of reduced LPL activity and increased VLDL production [38].

In this present study, no statistically significant differences in genotype distributions and allele frequencies of *APOC1* rs11568822 and *SORL1* rs2282649 polymorphisms were found between non-CAD and CAD groups. These results seem to be consistent with other research that found no association between *APOC1* rs11568822 polymorphism and either CAD or myocardial infarction [36]. In accordance with the present results, another study that investigates the association between sortilin levels, inflammation markers, and *SORL1* polymorphisms in coronary heart disease patients has demonstrated no difference in genotype distributions of rs2282649 polymorphism between groups [39]. In this study, no difference was found in allele frequencies and genotype distributions among CAD and non-CAD groups regarding *APOD*, *LIPA*, and *LDLR* polymorphisms. Although, this present study has a contribution to the literature and has importance since there were no previous studies to date that investigate the association between *APOD* rs1568565, *LIPA* rs13500, and *LDLR* rs5930 polymorphisms and CAD presence.

LAL deficiency as a result of the loss-of-function mutation in the *LIPA* gene was responsible for the lipid catabolism disorders such as Wolman disease and cholesteryl ester storage disease [40, 41]. In previous studies, an impaired ABCA1 protein expression along with the impaired cholesterol efflux was observed in LAL deficient individuals and these findings showed that LAL is one of the regulators of cholesterol efflux machinery and cholesterol homeostasis [42]. Also, in peritoneal macrophages isolated from lal$^{-/-}$ mice, cholesterol efflux was found in lower levels when compared to lal$^{+/+}$ mice [16]. In the present study, we observed that minor allele carriers of *LIPA* rs13500 polymorphism have higher levels of HDL-C in the non-CAD T2DM group. This finding could be a result of the increased expression of the *LIPA* gene. The rs13500 polymorphism is located on the 3' untranslated region of the gene and there are several microRNAs (miRNAs) that target this region according to prediction algorithms. The alteration of the miRNA binding site could affect the affinity of miRNA binding and therefore ultimately result in increased gene expression.

This present study is the first report that investigates the effects of the *APOD* rs1568565, *LIPA* rs13500, and *LDLR* rs5930 polymorphisms on CAD presence and disease risk factors. However, alongside the importance of the study, several limitations need to be considered. The effect of these five polymorphisms should be validated in a larger sample-sized study. In addition, there are no previous studies that investigate the functional effects of the polymorphisms and their implications on the expressions of the genes. Further research should be undertaken

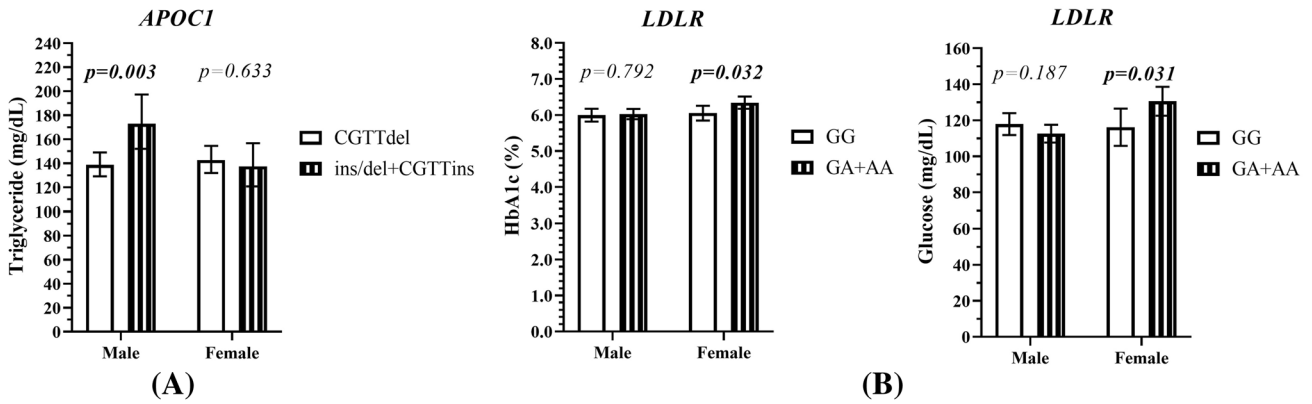

Fig. 2 Estimated mean and 95% confidence intervals of **A** triglyceride levels across the genotypes of *APOC1* rs11568822 in females and males, after adjustment for age, BMI, and usage of lipid-lowering drugs were given, triglyceride levels were found higher in rs11568822 minor allele carrier males. **B** Estimated mean and 95% confidence intervals of HbA1c and glucose levels in females and males were shown for *LDLR* rs5930 polymorphism. Both HbA1c and glucose levels were found higher in minor allele carrier females in the analysis adjusted to age, BMI, usage of lipid-lowering, and antidiabetic drugs

to investigate the possible mechanisms that underlie the findings of this study.

Conclusion

In conclusion, cholesterol-related gene polymorphisms influence lipid concentrations and the T2DM risk sex-specifically among Turkish CAD patients. We provided evidence that the *LDLR* rs5930 polymorphism is associated with T2DM presence only in the male CAD group. Besides, minor allele carrier females of *LDLR* polymorphism had elevated HbA1c and glucose levels independent from CAD status. In addition, the minor allele of *APOD* polymorphism was associated with decreased risk of T2DM in non-CAD. Also, *APOD*, *LIPA*, and *APOC1* gene polymorphisms were demonstrated to affect serum lipid levels. Due to the importance of these processes in atherosclerotic CAD, we can conclude that cholesterol-related gene polymorphisms may play a crucial role during atherogenesis. Therefore, further research is sorely warranted in this direction.

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval Whole sample collection and analysis processes were conducted in compliance with the ethical guidelines of the Declaration of Helsinki and approved by the Institutional Review Board at Istanbul University (Approval no: 377879).

Consent to participate The written informed consent was obtained from all participants and all experiments were performed in accordance with the approved guidelines and regulations.

References

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jiménez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB (2016) Executive summary: heart disease and stroke statistics–2016 update: a report from the american heart association. Circulation 133(4):447–454. https://doi.org/10.1161/cir.0000000000000366
2. Malakar AK, Choudhury D, Halder B, Paul P, Uddin A, Chakraborty S (2019) A review on coronary artery disease, its risk factors, and therapeutics. J Cell Physiol 234(10):16812–16823. https://doi.org/10.1002/jcp.28350
3. Lusis AJ (2000) Atherosclerosis Nature 407(6801):233–241. https://doi.org/10.1038/35025203
4. Navarro-Incio A, Tolivia-Fernández J (2004) The involvement of apolipoprotein D in pathologies affecting the nervous system. Rev Neurol 38(12):1166–1175
5. Rassart E, Bedirian A, Do Carmo S, Guinard O, Sirois J, Terrisse L, Milne R (2000) Apolipoprotein D. Biochim Biophys Acta

1482(1–2):185–198. https://doi.org/10.1016/s0167-4838(00)00162-x
6. Desai PP, Bunker CH, Ukoli FA, Kamboh MI (2002) Genetic variation in the apolipoprotein D gene among African blacks and its significance in lipid metabolism. Atherosclerosis 163(2):329–338
7. Jong MC, Hofker MH, Havekes LM (1999) Role of ApoCs in lipoprotein metabolism. Arterioscler Thromb Vasc Biol 19(3):472–484. https://doi.org/10.1161/01.ATV.19.3.472
8. McPherson A, Larson SB (2019) The structure of human apolipoprotein C-1 in four different crystal forms. J Lipid Res 60(2):400–411. https://doi.org/10.1194/jlr.M089441
9. Soutar AK, Naoumova RP (2007) Mechanisms of disease: genetic causes of familial hypercholesterolemia. Nat Clin Pract Cardiovasc Med 4(4):214–225
10. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, Hegele RA, Krauss RM, Raal FJ, Schunkert H, Watts GF, Borén J, Fazio S, Horton JD, Masana L, Nicholls SJ, Nordestgaard BG, van de Sluis B, Taskinen M-R, Tokgözoglu L, Landmesser U, Laufs U, Wiklund O, Stock JK, Chapman MJ, Catapano AL (2017) Low-density lipoproteins cause atherosclerotic cardiovascular disease 1 Evidence from genetic, epidemiologic, and clinical studies a consensus statement from the european atherosclerosis society consensus panel. Eur Heart J 38(32):2459–2472. https://doi.org/10.1093/eurheartj/ehx144
11. Catapano AL, Graham I, De BackerWiklund GO, Chapman MJ, Drexel H, Hoes AW, Jennings CS, Landmesser U, Pedersen TR, Reiner Ž, Riccardi G, Taskinen MR, Tokgozoglu L, Verschuren WMM, Vlachopoulos C, Wood DA, Zamorano JL, Cooney MT (2016) 2016 ESC/EAS guidelines for the management of dyslipidaemias. Eur Heart J 37(39):2999–3058. https://doi.org/10.1093/eurheartj/ehw272
12. Kanaki T, Bujo H, Hirayama S, Ishii I, Morisaki N, Schneider WJ, Saito Y (1999) Expression of LR11, a mosaic LDL receptor family member, is markedly increased in atherosclerotic lesions. Arterioscler Thromb Vasc Biol 19(11):2687–2695
13. Jiang M, Bujo H, Ohwaki K, Unoki H, Yamazaki H, Kanaki T, Shibasaki M, Azuma K, Harigaya K, Schneider WJ (2008) Ang II–stimulated migration of vascular smooth muscle cells is dependent on LR11 in mice. J Clin Invest 118(8):2733–2746
14. Suwa S, Ogita M, Takahashi N, Wada H, Dohi T, Kasai T, Okazaki S, Shimada K, Miyauchi K, Bujo H, Daida H (2020) Impact of LR11 as Residual risk on long-term clinical outcomes in patients with coronary artery disease treated with statins after first percutaneous coronary intervention. Int Heart J 61(3):470–475. https://doi.org/10.1536/ihj.19-686
15. Dubland JA, Francis GA (2015) Lysosomal acid lipase: at the crossroads of normal and atherogenic cholesterol metabolism. Front Cell Dev Biol 3:3. https://doi.org/10.3389/fcell.2015.00003
16. Du H, Schiavi S, Wan N, Levine M, Witte DP, Grabowski GA (2004) Reduction of atherosclerotic plaques by lysosomal acid lipase supplementation. Arterioscler Thromb Vasc Biol 24(1):147–154. https://doi.org/10.1161/01.ATV.0000107030.22053.1e
17. Gensini GG (1983) A more meaningful scoring system for determining the severity of coronary heart disease. Am J Cardiol 51(3):606. https://doi.org/10.1016/s0002-9149(83)80105-2
18. Serruys PW, Morice MC, Kappetein AP, Colombo A, Holmes DR, Mack MJ, Stahle E, Feldman TE, van den Brand M, Bass EJ, Van Dyck N, Leadley K, Dawkins KD, Mohr FW, Investigators S (2009) Percutaneous coronary intervention versus coronary-artery bypass grafting for severe coronary artery disease. N Engl J Med 360(10):961–972. https://doi.org/10.1056/NEJMoa0804626
19. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16(3):1215. https://doi.org/10.1093/nar/16.3.1215
20. Jamaldini SH, Babanejad M, Mozaffari R, Nikzat N, Jalalvand K, Badiei A, Sanati H, Shakerian F, Afshari M, Kahrizi K, Najmabadi H (2014) Association of polymorphisms at LDLR locus with coronary artery disease independently from lipid profile. Acta Med Iran 52(5):352–359
21. Jha CK, Mir R, Banu S, Elfaki I, Chahal SMS (2020) Heterozygosity in LDLR rs2228671 and rs72658855 gene is associated with increased risk of developing coronary artery disease in india -a case-control study. Endocr Metab Immune Disord Drug Targets 20(3):388–399. https://doi.org/10.2174/1871530319666191015164505
22. Salazar LA, Hirata MH, Giannini SD, Forti N, Diament J, Lima TM, Hirata RD (2000) Seven DNA polymorphisms at the candidate genes of atherosclerosis in Brazilian women with angiographically documented coronary artery disease. Clin Chim Acta 300(1–2):139–149. https://doi.org/10.1016/s0009-8981(00)00308-9
23. Guven G, Vurgun E, Bilgic B, Hanagasi H, Gurvit H, Ozer E, Lohmann E, Erginel-Unaltuna N (2019) Association between selected cholesterol-related gene polymorphisms and Alzheimer's disease in a Turkish cohort. Mol Biol Rep 46(2):1701–1707. https://doi.org/10.1007/s11033-019-04619-8
24. Zhu H, Tucker HM, Grear KE, Simpson JF, Manning AK, Cupples LA, Estus S (2007) A common polymorphism decreases low-density lipoprotein receptor exon 12 splicing efficiency and associates with increased cholesterol. Hum Mol Genet 16(14):1765–1772. https://doi.org/10.1093/hmg/ddm124
25. Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, Karczewski KJ, Karczewski KJ, Park J, Hitz BC, Weng S, Cherry JM, Snyder M (2012) Annotation of functional variation in personal genomes using RegulomeDB. Genome Res 22(9):1790–1797. https://doi.org/10.1101/gr.137323.112
26. Li C, Briggs MR, Ahlborn TE, Kraemer FB, Liu J (2001) Requirement of Sp1 and estrogen receptor alpha interaction in 17beta-estradiol-mediated transcriptional activation of the low density lipoprotein receptor gene expression. Endocrinology 142(4):1546–1553. https://doi.org/10.1210/endo.142.4.8096
27. Streicher R, Kotzka J, Müller-Wieland D, Siemeister G, Munck M, Avci H, Krone W (1996) SREBP-1 mediates activation of the low density lipoprotein receptor promoter by insulin and insulin-like growth factor-I. J Biol Chem 271(12):7128–7133. https://doi.org/10.1074/jbc.271.12.7128
28. He H, Yang F, Liu X, Zeng X, Hu Q, Zhu Q, Tu B (2007) Sex hormone ratio changes in men and postmenopausal women with coronary artery disease. Menopause 14(3 Pt 1):385–390. https://doi.org/10.1097/01.gme.0000235369.24479.dd
29. Ramakrishnan G, Arjuman A, Suneja S, Das C, Chandra NC (2012) The association between insulin and low-density lipoprotein receptors. Diab Vasc Dis Res 9(3):196–204. https://doi.org/10.1177/1479164111430243
30. Zhu L, Wu G, Yang X, Jia X, Li J, Bai X, Li W, Zhao Y, Li Y, Cheng W, Liu S, Jin S (2019) Low density lipoprotein mimics insulin action on autophagy and glucose uptake in endothelial cells. Sci Rep 9(1):3020. https://doi.org/10.1038/s41598-019-39559-7
31. Vijayaraghavan S, Hitman GA, Kopelman PG (1994) Apolipoprotein-D polymorphism: a genetic marker for obesity and hyperinsulinemia. J Clin Endocrinol Metab 79(2):568–570. https://doi.org/10.1210/jcem.79.2.7913935
32. Baker WA, Hitman GA, Hawrami K, McCarthy MI, Riikonen A, Tuomilehto-Wolf E, Nissinen A, Tuomilehto J, Mohan V, Viswanathan M et al (1994) Apolipoprotein D gene polymorphism: a new genetic marker for type 2 diabetic subjects in Nauru and south India. Diabet Med 11(10):947–952. https://doi.org/10.1111/j.1464-5491.1994.tb00252.x

33. Do Carmo S, Fournier D, Mounier C, Rassart E (2009) Human apolipoprotein D overexpression in transgenic mice induces insulin resistance and alters lipid metabolism. Am J Physiol Endocrinol Metab 296(4):E802-811. https://doi.org/10.1152/ajpendo.90725.2008
34. Jiménez-Palomares M, Cózar-Castellano I, Ganfornina MD, Sánchez D, Perdomo G (2011) Genetic deficiency of apolipoprotein D in the mouse is associated with nonfasting hypertriglyceridemia and hyperinsulinemia. Metabolism 60(12):1767–1774. https://doi.org/10.1016/j.metabol.2011.04.013
35. Zhang R, Liu Q, Liu H, Bai H, Zhang Y, Guan L, Fan P (2018) Effects of apoC1 genotypes on the hormonal levels, metabolic profile and PAF-AH activity in Chinese women with polycystic ovary syndrome. Lipids Health Dis 17(1):77. https://doi.org/10.1186/s12944-018-0725-5
36. Olsson B, Gigante B, Mehlig K, Bergsten A, Leander K, de Faire U, Lissner L, Thelle DS, Carlsson LM (2010) Apolipoprotein C-I genotype and serum levels of triglycerides, C-reactive protein and coronary heart disease. Metabolism 59(12):1736–1741. https://doi.org/10.1016/j.metabol.2010.04.017
37. Xu Y, Berglund L, Ramakrishnan R, Mayeux R, Ngai C, Holleran S, Tycko B, Leff T, Shachter NS (1999) A common Hpa I RFLP of apolipoprotein C-I increases gene transcription and exhibits an ethnically distinct pattern of linkage disequilibrium with the alleles of apolipoprotein E. J Lipid Res 40(1):50–58
38. Westerterp M, de Haan W, Berbée JF, Havekes LM, Rensen PC (2006) Endogenous apoC-I increases hyperlipidemia in apoE-knockout mice by stimulating VLDL production and inhibiting LPL. J Lipid Res 47(6):1203–1211. https://doi.org/10.1194/jlr.M500434-JLR200
39. Han WWZ, Zhang H, Geng C, Dang R, Yang M, Zhang J, Wang C, Jiang P (2020) The Association Between Sortilin and Inflammation in Patients with Coronary Heart Disease. J Inflamm Res 13:71–79. https://doi.org/10.2147/JIR.S240421
40. Bernstein DL, Hülkova H, Bialer MG, Desnick RJ (2013) Cholesteryl ester storage disease: review of the findings in 135 reported patients with an underdiagnosed disease. J Hepatol 58(6):1230–1243. https://doi.org/10.1016/j.jhep.2013.02.014
41. Zhang H (2018) Lysosomal acid lipase and lipid metabolism: new mechanisms, new questions, and new therapies. Curr Opin Lipidol 29(3):218–223. https://doi.org/10.1097/mol.0000000000000507
42. Bowden KL, Bilbey NJ, Bilawchuk LM, Boadu E, Sidhu R, Ory DS, Du H, Chan T, Francis GA (2011) Lysosomal acid lipase deficiency impairs regulation of ABCA1 gene and formation of high density lipoproteins in cholesteryl ester storage disease. J Biol Chem 286(35):30624–30635. https://doi.org/10.1074/jbc.M111.274381