CYPRUS JOURNAL OF MEDICAL SCIENCES

Original Article

DOI: 10.5152/cjms.2018.531

Assessment of the Association Between Three Perplexing *PPARA* Gene Polymorphisms and the Risk of Coronary Artery Disease in a Population of Turkish Cypriot Women

Mahmut Çerkez Ergören[1], Kerem Teralı[2]

[1]Department of Medical Biology, Near East University School of Medicine, Nicosia, Cyprus
[2]Department of Medical Biochemistry, Near East University School of Medicine, Nicosia, Cyprus

ORCID IDs of the authors: M.Ç.E. 0000-0001-9593-9325; K.T. 0000-0002-9964-6383.

Cite this article as: Ergören MÇ, Teralı K. Assessment of the Association Between Three Perplexing PPARA Gene Polymorphisms and the Risk of Coronary Artery Disease in a Population of Turkish Cypriot Women. Cyprus J Med Sci 2018; 3: 75-80.

BACKGROUND/AIMS
Cardiovascular diseases, particularly coronary artery disease (CAD) and myocardial infarction, are the most significant causes of mortality among people worldwide. CAD is enormously complex in its interplay of environment and genetics, with numerous genetic loci contributing to its heritability. Peroxisome proliferator-activated receptor α (PPARα) is a transcription factor that is activated by physiological, pharmacological, or nutritional stimulation and acts to modulate lipid metabolism by regulating the expression of its target genes. Here, we aim to investigate the potential pleiotropic effects of three *PPARA* polymorphic loci (rs4253778, rs1800206, and rs135539) on CAD risk in a population of Turkish Cypriot women and also whether these effects are mediated by plasma lipid concentrations in the same population.

MATERIALS and METHODS
A total of 62 women with CAD and 117 otherwise healthy women were included in this population-based case–control study. Genomic DNA was extracted from peripheral blood samples, and the relevant *PPARA* polymorphisms were determined by the restriction endonuclease analysis of amplicons generated by polymerase chain reaction.

RESULTS
Genotyping and subsequent case–control comparisons revealed that although there was no over- or under-representation of the six tested *PPARA* alleles in the disease state, the homozygous presence of the *PPARA* rs4253778 C allele (genotype CC) was associated with increased triglyceride concentrations in patients with CAD (p=0.005).

CONCLUSION
Along with other yet-to-be-ascertained susceptibility loci, the *PPARA* rs4253778 polymorphic locus may be employed in risk stratification in community-level screening for CAD among Turkish Cypriot women.

Keywords: Coronary artery disease, peroxisome proliferator-activated receptors, *PPARA* polymorphisms, genetic epidemiology, Turkish Cypriot women

INTRODUCTION

According to the estimates of the World Health Organization (1), cardiovascular diseases are the number one cause of mortality worldwide, accounting for 17.7 million deaths in 2015. These numbers correspond to 31% of all global deaths, of which an estimated 7.4 million were due to coronary heart disease, a result of coronary artery disease (CAD). CAD refers to a narrowing/constriction of the coronary arteries supplying myocardial demands for oxygen and nutrients through a process in which fatty plaque gradually accumulates in the intima of the artery (atherosclerosis). The rupture of vulnerable plaques with active inflammation is responsible for coronary thrombosis-the major cause of acute myocardial infarction (2). As with nearly all complex diseases, CAD stands out for the strong interplay of lifestyle and genetic factors, with more than 50 genetic loci linked to coronary risk (3).

Peroxisome proliferator-activated receptors (PPARs) are a family of transcription factors that are activated by several natural and synthetic ligands. In molecular and cellular context, PPARs belong to the superfamily of nuclear hormone

Corresponding Author: Kerem Teralı
E-mail: kerem.terali@neu.edu.tr

Received: 14.05.2018
Accepted: 27.06.2018

receptors that include thyroid hormone receptor, steroid hormone receptors, vitamin D_3 receptor, and retinoic acid receptor, among others (4). PPARs act through genomic mechanisms as well as through mechanisms that do not involve a genomic component. Additionally, their activity is subject to regulation by posttranslational modifications, such as phosphorylation, SUMOylation, and ubiquitination (5). PPAR family constitutes three subtypes, each encoded by distinct but homologous genes: PPARα (encoded by *PPARA*), PPARβ/δ (encoded by *PPARB/D*), and PPARγ (encoded by *PPARG*). They differ from each other in terms of tissue distribution, ligand specificity, and normal function. PPARα is significantly expressed in metabolically active tissues (e.g., skeletal muscle, heart, liver, brown fat, and intestinal mucosa) and, upon physiological, pharmacological, or nutritional activation, modulates lipid metabolism via the regulation of numerous target genes (6). Several polymorphisms in both the coding and noncoding regions of the *PPARA* locus contribute to dyslipidemias and imply metabolic and cardiovascular risks (7). The three most intensively studied polymorphic loci in the *PPARA* gene include rs4253778 (a G/C transversion in intron 7), rs1800206 (a C/G transversion at position 484 in exon 5), and rs135539 (an A/C transversion in intron 1).

Data from a study involving a cohort of Finnish men with coronary artery bypass graft and low plasma high-density lipoprotein-cholesterol (HDL-C) led to the conclusion that the *PPARA* rs1800206 G allele was associated with a decreased progression of coronary atherosclerosis, whereas the *PPARA* rs4253778 C allele was associated with increased progression of the disease (8). Moreover, the atheroprotective rs1800206 G allele was shown to greatly reduce the proatherosclerotic effect of the rs4253778 C allele. Interestingly, the action of these two *PPARA* gene polymorphisms was found not to be mediated via plasma lipid concentrations. Moreover, the interrelationship between dyslipidemia and the rs4253778 G/C polymorphism was absent in 820 subjects of Chinese Han origin or descent (9). Mazotti et al. (10), however, have revealed a discrepancy in the association of the rs4253778 G/C polymorphism with dyslipidemia in a Brazilian population. The pattern of this discrepancy was such that while the rs4253778 C allele was associated with high HDL-C levels and low triglyceride (TG) and plasma low-density lipoprotein-cholesterol (LDL-C) levels in subjects from São Paulo City (11), the same allele was associated with dyslipidemia in subjects from Cuiaba City (10). In white Polish Caucasian patients with CAD compared with healthy blood donors of the same ethnic origin, the proatherosclerotic *PPARA* rs4253778 C allele carrier state was associated with the disease in male subgroups (12).

The *PPARA* rs135539 A→C variant was shown to be significantly associated with the incidence of low (<40 mg dl^{-1}) plasma HDL-C levels in a Chinese population from Jiangsu Province (13). In the aforementioned study from China, the same variant was also found to interact with the *PPARA* rs1800206 C/G polymorphism as well as several *PPARG* polymorphisms. The pathological significance of the *PPARA* rs1800206 C/G polymorphism for the alteration of lipid profile was shown by both SNP and haplotype association tests, through which the causal role of the *PPARA* rs1800206 C/G polymorphism (either alone or in interaction with the other tested *PPARA/PPARD/PPARG* polymorphisms) in hypertriglyceridemia was ascertained (14). In addition, the two genotypes with the minor allele variants at the *PPARA* rs1800206 locus (CG+GG) were demonstrated to be associated with elevated LDL-C levels in a Chinese Han population (15). Significant gene-gene interactions among various PPAR gene polymorphisms were also proposed by the same research group to contribute to an increased risk of cardiovascular disease and atherosclerosis. In another study from Jiangsu Province, China, researchers concluded that compared with subjects homozygous for the wild-type genotype (CC), subjects carrying the minor allele of the *PPARA* rs1800206 C/G polymorphism showed significantly higher plasma lipoprotein(a) [Lp(a)] levels and that the *PPARA* rs1800206 C/G polymorphism interacted with the *PPARA* rs135539 A/C polymorphism as well as with more than a few *PPARG* polymorphisms (16). Taken together, these data indicate that rs135539 and rs1800206 represent the two functional polymorphic loci in the *PPARA* gene and that genetic variation at these loci modulates lipid profile, at least in people of Chinese Han origin or descent.

The developing population of North Cyprus has a noticeably higher prevalence of hypercholesterolemia/dyslipidemia and cardiovascular diseases, which could possibly be due to increased adoption of sedentary lifestyle and unhealthy dietary habits and/or with the restricted genetic pool of the community (17). Therefore, this study aimed to investigate the potential nontraditional roles of the *PPARA* rs4253778 G/C, rs1800206 C/G, and rs135539 A/C polymorphisms in susceptibility to CAD in a population of Turkish Cypriot women and also to examine whether these effects are mediated by plasma lipid levels in the same population.

MATERIALS and METHODS

Subjects, Study Design and Biochemical Analysis

The current study included 179 unrelated women volunteers from the Turkish Cypriot population. Each subject was provided with a questionnaire through which data on demographic characteristics, including age, ethnicity, socioeconomic background, and general health status were collected. The Turkish Cypriot ethnicity was defined as residing in North Cyprus as well as being born to parents who have been living in the island of Cyprus for at least two generations. In total, 62 women, who were diagnosed with CAD by a cardiologist according to the most recent national guidelines of the Turkish Society of Cardiology, constituted the CAD group, while 117 otherwise healthy (i.e., no evidence of type 2 diabetes, hypertension, obesity, hypercholesterolemia, or family/previous history of stroke or transient ischemic attacks) women constituted the control group. The non-CAD subjects were matched according to their age and socioeconomic background. Current smokers were excluded from both groups, whereas subjects under treatment with statins and/or anticoagulants were included in the CAD group. Antecubital venous blood from the subjects was collected in tubes containing ethylenediaminetetraacetate (EDTA) and subjected to centrifugation within 2 h of collection. The fasting levels of plasma glucose, serum total cholesterol, HDL-C, LDL-C, and TG were measured using an automatic biochemical analyzer in the medical biochemistry laboratory of our university hospital. The study protocol was approved by the Ethics Committee of our university (application no. NEU/2016/36/282), and all subjects gave written informed consent to participate in the study.

TABLE I. Details of PCR primers and restriction endonucleases used and of the resulting restriction fragments (Note: the mismatch nucleotide in the reverse primer for rsI800206 is underlined)

PPARA polymorphic locus	PCR primers (from 5′ to 3′)	Restriction enzyme	Digestion pattern	Reference
rs4253778	F: ACAATCACTCCTTAAATATGGTGG	*Taq*I	G allele: 266 bp	(8)
	R: AAGTAGGGACAGACAGGACCAGTA		C allele: 216 bp, 50 bp	
rsI800206	F: GACTCAAGCTGGTGTATGACAAGT	*Hinf*I	C allele: 117 bp	(18)
	R: CGTTGTGTGACATCCCGACAGAAT		G allele: 93 bp, 24 bp	
rsI35539	F: CCAGGGGGAGGAAAGAGTGAA	*Hinf*I	A allele: 210 bp	(19)
	R: GCCACAACTAAGCAGGCAGTG		C allele: 148 bp, 62 bp	

F: forward primer; R: reverse primer; PCR: polymerase chain reaction; PPARA: peroxisome proliferator-activated receptors

TABLE 2. Characteristics of the subjects

	Groups		
Variable[a]	Control (n=117)	CAD (n=62)	p (two-tailed)
Age (years)	43.7±11.2	40.0±11.4	0.752 (95% CI=-0.430 to 8.820)
Glucose (mg dL^{-1})	95.0±11.7	93.4±26.7	0.732 (95% CI=-8.140 to 11.433)
Cholesterol (mg dL^{-1})	188.5±52.0	195.4±40.6	0.511 (95% CI=-27.482 to 13.775)
HDL-C (mg dL^{-1})	56.0±12.5	50.5±10.3	0.034* (95% CI=-0.418 to 10.746)
LDL-C (mg dL^{-1})	126.3±29.4	127.2±44.7	0.922 (95% CI=-18.495 to 16.760)
TG (mg dL^{-1})	95.321±35.5	110.2±41.7	0.100 (95% CI=-32.870 to 2.954)

CAD: coronary artery disease; N: number of subjects; CI: confidence interval; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; TG: triglyceride; a: values are represented as mean±standard deviation; *: statistical significance

Genotyping

Genomic DNA of each subject was extracted from EDTA-treated whole blood using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The genotyping of the *PPARA* rs4253778 G/C, rsI800206 C/G, and rsI35539 A/C polymorphisms was performed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The details of PCR primers, restriction enzymes, and digestion patterns are shown in Table I (8, 18, 19). PCR amplification was performed on a conventional thermal cycler (Corbett Research Pty. Ltd., Sydney, Australia) with a total volume of 20 µl containing 1× Taq Buffer (ThermoFisher Scientific, Waltham, CA, USA), 0.05 U μl^{-1} Taq DNA Polymerase (ThermoFisher Scientific), 1.5 mM $MgCl_2$ (ThermoFisher Scientific), 0.2 mM dNTPs (abm, Inc., Vancouver, CA, USA), 10 nM of each primer (İntron Sağlık Ürünleri İth. İhr. Tic. Ltd. Şti., İzmir, Turkey), and approximately 50 ng of the genomic DNA template. A class II laminar flow hood, designated pipettes, PCR-clean reagents and consumables, and ultra-violet (UV)-treated solutions were employed to minimize the risk of contamination during genomic DNA extraction and PCR amplification. Digestion products were separated on 3% agarose gels and visualized by ethidium bromide staining and subsequent UV transillumination. Genotypes were assigned on the basis of the presence or absence of restriction sites.

Statistical Analysis

The data were processed to express the continuous variables that were normally distributed as group mean±standard deviation. Intergroup differences in continuous variables were assessed by Student's unpaired t-test, where a $p<0.05$ (two-tailed) was considered statistically significant. Genotype distributions and allele frequencies were calculated by the gene-counting method and their compliance to the Hardy–Weinberg equilibrium was evaluated by the goodness-of-fit χ^2 test, where a $p<0.05$ was considered to indicate significant disequilibrium. The association between the case–control status and each polymorphism was assessed by the odds ratio and its corresponding 95% confidence interval (CI), where a $p<0.05$ was considered statistically significant. The influence of the assigned genotypes on biochemical parameters was evaluated using one-way analysis of variance (ANOVA) for each polymorphism. Statistical significance was set at $p<0.05$. The aforementioned single-locus data analyses were performed using the commercial GraphPad Prism software (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

The demographic characteristics and biochemical parameters of the subjects, from whom blood samples were obtained, are shown in Table 2. The subjects comprised 179 Turkish Cypriot women, including 62 women with CAD and 117 otherwise healthy women (i.e., the control group). The CAD group showed no statistically significant difference from the control group in terms of age; fasting plasma glucose levels; and serum concentrations of total cholesterol, LDL-C, and TG, while the serum concentrations of HDL-C were significantly lower in the CAD group than in the control group (p=0.034; 95% CI=-0.418 to 10.746). It should be noted, however, that serum concentrations of HDL-C in the CAD group (50.5±10.3 mg dl^{-1}) fall within the normal range of 40-59 mg dl^{-1} (20), which is neither a major risk factor for heart disease nor considered protective against heart disease.

Genotype distributions and allele frequencies for the *PPARA* rs4253778, rsI800206, and rsI35539 polymorphisms among the

TABLE 3. Genotype distributions and allele frequencies for the *PPARA* rs4253778 G/C, rsI800206 C/G, and rsI35539 A/C polymorphisms among the CAD and control groups

***PPARA* rs4253778 G/C**										
	Genotype distribution					**Allele frequency**				
Group	**GG**	**GC**	**CC**	**X^2**	**p**	**G**	**C**	**p**	**OR**	**95% CI**
Control (n=117)	80 (68.0%)	33 (28.0%)	4 (4.0%)	0.070	0.791	0.820	0.180	0.959	0.983	0.520 to 1.858
CAD (n=62)	43 (70.0%)	16 (26.0%)	3 (4.0%)	0.030	0.362	0.830	0.170			
***PPARA* rsI800206 C/G**										
	Genotype distribution					**Allele frequency**				
Group	**CC**	**CG**	**GG**	**X^2**	**p**	**C**	**G**	**p**	**OR**	**95% CI**
Control (n=117)	109 (93.2%)	8 (6.8%)	0 (0%)	0.150	0.698	0.960	0.040	0.926	0.934	0.273 to 3.257
CAD (n=62)	58 (94.0%)	4 (6.0%)	0 (0%)	0.070	0.791	0.970	0.030			
***PPARA* rsI35539 A/C**										
	Genotype distribution					**Allele frequency**				
Group	**AA**	**AC**	**CC**	**X^2**	**p**	**A**	**C**	**p**	**OR**	**95% CI**
Control (n=117)	52 (44.5%)	59 (50.5%)	6 (5.0%)	4.360	0.036	0.700	0.300	0.963	1.280	0.773 to 2.119
CAD (n=62)	20 (32.3%)	36 (58.0%)	6 (8.7%)	3.100	0.078	0.610	0.390			

CAD: coronary artery disease; n: number of subjects; OR: odds ratio; CI: confidence interval; PPARA: peroxisome proliferator-activated receptors

62 patients and 117 controls are shown in Table 3. Distributions of the *PPARA* rs4253778 and rsI800206 genotypes were in compliance with the Hardy–Weinberg equilibrium (p>0.050). The *PPARA* rsI35539 genotypes in the control group, however, appeared to slightly deviate from the Hardy–Weinberg equilibrium (p=0.036), indicating that possibly a selective dropout of a given allele occurred. The frequencies of the minor alleles *PPARA* rs4253778 C, rsI800206 G, and rsI35539 C among the control group were 0.170, 0.030, and 0.390, respectively.

Case–control genetic association analysis revealed that no significant difference in the allele frequencies of the three polymorphisms of interest between the patients and healthy subjects. Among all subjects, the *PPARA* rs4253778 G/C marker was found to be significantly associated with TG levels in the CAD group (p=0.005) (Table 4). Accordingly, the CC genotype seemed to increase serum concentrations of TG to borderline high levels of 150-199 mg dl^{-1} (20).

DISCUSSION

Here, we attempted to investigate the association of the *PPARA* rs4253778 G/C, rsI800206 C/G, and rsI35539 A/C polymorphisms with the risk of CAD in a population of white women of Turkish Cypriot origin. To our knowledge, this is the first study in the relevant scientific literature to investigate the *PPARA* gene polymorphisms in this population, providing a better understanding of the predisposition of Turkish Cypriot women to CAD. Today, the Turkish Cypriot community is concentrated in the Turkish Cypriot-administrated North Cyprus. With its 3,355 km^2, North Cyprus is a relatively small land area with a de jure population of only 286,257 (of which 47.4% constitute women) (21). In a genealogical context, the Turkish Cypriot paternal lineages were shown to have an autochthonous character and the closest genetic ties to the neighboring Near Eastern populations (22).

Coronary artery disease denotes the build-up of atherosclerotic plaque in the major blood vessels that supply oxygen and nutrients to the heart. The accumulated data have demonstrated that elevated levels of circulating LDL-C, elevated TG-rich lipoproteins, or reduced HDL-C are associated with the risk of CAD and that genetic loci involved in the causal pathways have robust associations with the risk of CAD in a similar manner (3). Given its role in modulating serum lipid levels and exerting direct atherogenic effects at the vascular wall level (23), *PPARA* could be a causal gene for CAD. In our study, the frequencies of the minor alleles *PPARA* rs4253778 C, rsI800206 G, and rsI35539 C among the non-CAD women were closely comparable to those reported among the European population (24). Although our findings failed to reveal a significant over- or under-representation of the six *PPARA* alleles of interest in the disease state, they revealed that the homozygous presence of the *PPARA* rs4253778 C allele (genotype CC) was associated with increased TG levels in the CAD group. Several target genes involved in lipid metabolism are regulated by the PPARα transcription factors. As just two of several examples, PPARα agonists induce liver β-oxidative enzymes, which shift free fatty acid metabolism from TG synthesis to degradation, while PPARα activators increase the activity of lipoprotein lipase, which catalyzes TG degradation and lowers plasma TG-rich lipoprotein levels (25). It is appealing to speculate, here, that the CC genotype could possibly impair TG metabolism in CAD.

Our data are partly in agreement with those obtained by others; Mazotti et al. (10) found a significant association between the *PPARA* rs4253778 C allele and dyslipidemia (which was defined as the use of oral hypolipemiants or as total cholesterol>200 mg dl^{-1}, HDL-C<40 mg dl^{-1}, LDL-C>130 mg dl^{-1}, and TG>150 mg dl^{-1}) in a Brazilian population from Cuiaba City.

TABLE 4. Effects of the assigned genotypes on the CAD and control groups' biochemical characteristics

***PPARA* rs4253778 G/C**

Control group (n=117)					CAD group (n=62)				
Biochemical parameter[a] (in mg dl^{-1})	Genotype			ANOVA p	Biochemical parameter (in mg dl^{-1})	Genotype			ANOVA p
	GG	GC	CC			GG	GC	CC	
Glucose	95.2±32.2	89.6±9.3	92.3±14.9	0.727	Glucose	93.9±8.4	97.6±17.2	90.4±4.5	0.560
Cholesterol	195.4±8.3	174.1±47.0	183.4±40.4	0.332	Cholesterol	190.8±37.1	207.6±49.1	236.6±15.2	0.141
HDL-C	55.2±12.5	57.5±13.7	58.6±2.8	0.779	HDL-C	50.8±10.7	49.3±8.1	64.4±21.8	0.130
LDL-C	126.9±43.5	116.6±36.7	130.4±53.1	0.667	LDL-C	121.1±27.7	139.1±31.7	154.0±34.1	0.104
TG	104.4±38.2	115.7±44.9	119.4±44.2	0.557	TG	91.6±36.2	103.5±35.4	169.6±15.3	0.005*

***PPARA* rs1800206 C/G**

Control group (n=117)					CAD group (n=62)				
Biochemical parameter (in mg dl^{-1})	Genotype			ANOVA p	Biochemical parameter (in mg dl^{-1})	Genotype			ANOVA p
	CC	CG	GG			CC	CG	GG	
Glucose	93.5±31.4	105.5±7.0	-	0.600	Glucose	93.9±11.2	92.5±3.5	-	0.865
Cholesterol	177.3±50.5	247.5±23.3	-	0.058	Cholesterol	202.1±46.6	208.5±33.0	-	0.794
HDL-C	59.7±12.3	59.5±1.5	-	1.000	HDL-C	59.3±8.2	64.7±9.9	-	0.294
LDL-C	116.0±40.5	170.5±28.9	-	0.680	LDL-C	122.8±41.5	144.4±37.7	-	0.368
TG	108.9±38.6	150.5±105.3	-	0.175	TG	106.1±38.6	124.5±24.5	-	0.400

***PPARA* rs135539 A/C**

Control group (n=117)					CAD group (n=62)				
Biochemical parameter (in mg dl^{-1})	Genotype			ANOVA p	Biochemical parameter (in mg dl^{-1})	Genotype			ANOVA p
	AA	AC	CC			AA	AC	CC	
Glucose	90.8±9.4	96.5±34.5	83.2±4.7	0.480	Glucose	100.6±16.9	101.1±22.8	101.8±12.3	0.980
Cholesterol	194.4±60.0	189.9±45.4	177.5±16.3	0.811	Cholesterol	201.8±45.7	202.0±40.2	187.5±40.1	0.772
HDL-C	57.3±12.1	53.3±12.6	59.7±13.5	0.369	HDL-C	47.3±5.3	49.7±11.4	55.9±14.1	0.219
LDL-C	128.1±48.0	127.5±34.5	95.6±26.0	0.411	LDL-C	137.7±29.2	128.8±26.0	111.2±35.8	0.190
TG	111.3±38.4	121.2±40.1	104.7±54.2	0.951	TG	138.5±16.1	95.8±6.8	74.7±2.7	0.352

CAD: coronary artery disease; n: number of subjects; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; TG: triglyceride; a: values are represented as mean±standard deviation; *: statistical significance

However, the relevant scientific literature also provides genetic epidemiological evidence of either a protective effect or a lack of effect of the *PPARA* rs4253778 C allele in other populations (8, 9, 11). Besides genetic composition, lifestyle, physical activity, and dietary patterns also substantially vary among different populations, giving a reasonable explanation for the apparent discrepancy between our study and the other studies.

As with other genetic association studies, the present study has several limitations. First, the number of women included in our study is relatively small, which lowers the statistical power. Second, the rs4253778 G/C and rs135539 A/C polymorphisms are located within the intronic regions of the *PPARA* gene, and this confronts us with the challenge of precisely describing their functional relevance. Third, the epistatic interactions between the *PPARA* variants and other genes and also *PPARA*–environment interactions remain to be thoroughly characterized, and this makes it difficult to draw definite conclusions about the causal relationship between the *PPARA* polymorphisms and the risk of CAD.

Overall, the results from our study establish the homozygous wild-type genotype (CC) at the *PPARA* rs4253778 locus as a genetic risk factor with a TG-elevating effect in a population of Turkish Cypriot women with CAD. Along with other yet-to-be-determined genetic loci, the *PPARA* rs4253778 polymorphic locus may be offered as a screening option to women who come in for medical check-up, and, depending on the results, they may be encouraged to make lifestyle modifications, such as ceasing smoking, eating a healthier diet, and engaging in more exercise. It is also worth mentioning that although candidate gene association studies of CAD remain an effective means of defining common variants and individually testing each variant by comparing its frequency in affected patients and healthy subjects, the relatively small number of inhabitants of North Cyprus calls for genome-wide association studies of CAD and other cardiovascular diseases in the Turkish Cypriot population.

Ethics Committee Approval: Ethics committee approval was received from the ethics committee of Near East University (Approval Date: 31.03.2016; Approval Number: NEU/2016/36/282).

Informed Consent: Written informed consent was obtained from all participants who participated in this study

Peer-review: Externally peer-reviewed.

Author contributions: Concept - K.T., M.E.; Design - K.T., M.E.; Supervision - K.T., M.E.; Resource - M.E.; Materials - M.E.; Data Collection and/or Processing - K.T., M.E.; Analysis and/or Interpretation - K.T., M.E.; Literature Search - K.T.; Writing - K.T.; Critical Reviews - K.T., M.E.

Acknowledgements: The authors acknowledge the financial support from Near East University as well as the assistance with patient selection from the Near East University Hospital's cardiovascular surgery outpatient clinics.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

REFERENCES

1. World Health Organization. Cardiovascular diseases (CVDs) [Fact sheet]. 2017. (Access: 20.01.2018). Available from: http://www.who.int/mediacentre/factsheets/fs317/en/.
2. Libby P. The vascular biology of atherosclerosis. In: Bonow RO, Mann DL, Zipes DP, Libby P, editors. Braunwald's heart disease: a textbook of cardiovascular medicine, 10th edn. Saunders, Massachusetts; 2014. p 873-90.
3. Khera AV, Kathiresan S. Genetics of coronary artery disease: discovery, biology and clinical translation. Nat Rev Genet 2017; 18: 331-44. [CrossRef]
4. Grygiel-Górniak B. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications-a review. Nutr J 2014; 13: 17. [CrossRef]
5. Youssef JA, Badr MZ. Peroxisome proliferator-activated receptors: discovery and recent advances. Springer ScienceδBusiness Media, New York; 2013. [CrossRef]
6. Kersten S. Integrated physiology and systems biology of PPARα. Mol Metab 2014; 3: 354-71. [CrossRef]
7. Yong EL, Li J, Liu MH. Single gene contributions: genetic variants of peroxisome proliferator-activated receptor (isoforms α, β/δ and γ) and mechanisms of dyslipidemias. Curr Opin Lipidol 2008; 19: 106-12. [CrossRef]
8. Flavell DM, Jamshidi Y, Hawe E, Pineda Torra I, Taskinen MR, Frick MH, et al. Peroxisome proliferator-activated receptor α gene variants influence progression of coronary atherosclerosis and risk of coronary artery disease. Circulation 2002; 105: 1440-5. [CrossRef]
9. Gu SJ, Guo ZR, Zhou ZY, Hu XS, Wu M. PPAR α and PPAR γ polymorphisms as risk factors for dyslipidemia in a Chinese Han population. Lipids Health Dis 2014; 13: 23. [CrossRef]
10. Mazzotti DR, Singulane CC, Ota VK, Rodrigues TP, Furuya TK, de Souza FJ, et al. PPARα polymorphisms as risk factors for dyslipidemia in a Brazilian population. Mol Genet Metab 2011; 102: 189-93. [CrossRef]
11. Chen ES, Mazzotti DR, Furuya TK, Cendoroglo MS, Ramos LR, Araujo LQ, et al. Association of PPARα gene polymorphisms and lipid serum levels in a Brazilian elderly population. Exp Mol Pathol 2010; 88: 197-201. [CrossRef]
12. Żak I, Balcerzyk A, Sarecka B, Niemiec P, Ciemniewski Z, Dyląg S. Contemporaneous carrier-state of two or three "proatherosclerotic" variants of APOE, ICAM1, PPARA and PAI-1 genes differentiate CAD patients from healthy individuals. Clin Chim Acta 2005; 362: 110-8. [CrossRef]
13. Liu MM, Gu SJ, Guo ZR, Wu M, Chen Q, Zhou ZY, et al. Association and interaction of peroxisome proliferator-activated receptor α with low high-density lipoprotein hyperlipidemia and with peroxisome proliferator-activated receptor γ. Zhonghua Liu Xing Bing Xue Za Zhi 2012; 33: 1218-23.
14. Gu SJ, Liu MM, Guo ZR, Wu M, Chen Q, Zhou ZY, et al. Gene-gene interactions among PPARα/δ/γ polymorphisms for hypertriglyceridemia in Chinese Han population. Gene 2013; 515: 272-6. [CrossRef]
15. Fan W, Shen C, Wu M, Zhou ZY, Guo ZR. Association and interaction of PPARα, δ, and γ gene polymorphisms with low-density lipoprotein-cholesterol in a Chinese Han population. Genet Test Mol Biomarkers 2015; 19: 379-86. [CrossRef]
16. Xie HJ, Hai B, Wu M, Chen Q, Liu MM, Dong C, et al. Analysis on the association between PPARα/γ polymorphisms and lipoprotein(a) in a Chinese Han population. Mol Genet Genomics 2014; 289: 981-7. [CrossRef]
17. Fahrioğlu U, Ergören MÇ. The association between APOA5 gene polymorphisms and plasma lipids in the Turkish Cypriot population: A Possible biomarker for preventing cardiovascular diseases. Biochem Genet 2018; 56: 176-87. [CrossRef]
18. Vohl MC, Lepage P, Gaudet D, Brewer CG, Bétard C, Perron P, et al. Molecular scanning of the human PPARα gene: association of the L162V mutation with hyperapobetalipoproteinemia. J Lipid Res 2000; 41: 945-52.
19. Flavell DM, Ireland H, Stephens JW, Hawe E, Acharya J, Mather H, et al. Peroxisome proliferator-activated receptor α gene variation influences age of onset and progression of type 2 diabetes. Diabetes 2005; 54: 582-6. [CrossRef]
20. National Cholesterol Education Program Expert Panel. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. Circulation 2002; 106: 3143-421.
21. TRNC State Planning Organization. [2011 Census Results]. 2011. (Access: 27.01.2018.) Available from: http://www.devplan.org/Frame-tr.html.
22. Gurkan C, Sevay H, Demirdov DK, Hossoz S, Ceker D, Teralı K, et al. Turkish Cypriot paternal lineages bear an autochthonous character and closest resemblance to those from neighbouring Near Eastern populations. Ann Hum Biol 2017; 44: 164-74. [CrossRef]
23. Barbier O, Torra IP, Duguay Y, Blanquart C, Fruchart JC, Glineur C, et al. Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis. Arterioscler Thromb Vasc Biol 2002; 22: 717-26. [CrossRef]
24. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM et al. A global reference for human genetic variation. Nature 2015; 526: 68-74. [CrossRef]
25. van Raalte DH, Li M, Pritchard PH, Wasan KM. Peroxisome proliferator-activated receptor (PPAR)-α: a pharmacological target with a promising future. Pharm Res 2004; 21: 1531-8. [CrossRef]