Case-control study on *PCSK9 R496W* (rs374603772) and *D374Y* (rs137852912) mutations in Turkish patients with primary dyslipidemia

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Abstract

Objective: The aim of this study was to investigate the relationships between *F216L* (rs28942112), *R496W* (rs374603772), *S127R* (rs28942111), and *D374Y* (rs137852912) *PCSK9* gain-of-function (GOF) mutations and primary dyslipidemia and serum lipid levels in patients with primary dyslipidemia.

Methods: In this case-control study, DNA was isolated from blood samples collected from patients diagnosed with primary dyslipidemia in cardiology outpatient clinic of Ege University (n=200) and healthy individuals (n=201). *F216L*, *R496W*, *S127R*, and *D374Y* GOF mutations in the *PCSK9* gene were evaluated and genotyped according to the results of melting curve analysis performed in a real-time polymerase chain reaction (PCR) 480 instrument using specific primers for each mutation.

Results: There were statistically significant differences between the patient and individuals in control groups in the *R496W* and *D374Y* mutations (χ^2=10.742 p=0.005; χ^2=6.078 p=0.048, respectively). In addition, triglyceride levels in patients with primary dyslipidemia heterozygous for *R496W* and *D374Y* mutations were 12.8-fold (p=0.015) and 3.4-fold (p=0.03) higher than that in mutant and wild-type genotype, respectively. Additionally, in the entire study group (n=401), *PCSK9 R496W* and *D374Y* mutation carriers had increased total cholesterol (p=0.021), triglycerides (p=0.0001), HDL cholesterol (p=0.028), and low-density lipoproteins (LDL) cholesterol (p=0.028) levels. However, *F216L* (rs28942112) and *S127R* (rs28942111) mutations were not detected in patients with primary dyslipidemia and healthy controls.

Conclusion: We conclude that the *PCSK9 R496W* (rs374603772) and *D374Y* (rs137852912) GOF mutations may be significant risk factors in the development of primary dyslipidemia and may have significant impact on lipid parameters in general population. *(Anatol J Cardiol 2018; 19: 334-40)*

Keywords: primary dyslipidemia, *PCSK9, R496W, D374Y*

Introduction

Dyslipidemia is defined as a functional disorder resulting in increased or decreased levels of lipoproteins (1). Primary dyslipidemias are lipid metabolism disorders caused by mutations in genes encoding proteins that play an important role in lipoprotein metabolism (2). Clinically, the most common dyslipidemias are characterized by elevated beta-lipoproteins [very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL)], with or without hypertriglyceridemia. Increased serum concentrations of LDL and other lipoproteins and lipid fractions are directly associated with the development of atherosclerosis and cardiovascular disease (CVD) (1). LDL cholesterol (LDL-C) that accumulates in the vascular wall and triggers immunological and oxidative processes plays an important role in the pathology of atherosclerosis (3-7).

Studies have shown that genetic factors are responsible for a significant proportion of the variations in lipid profiles and that changes in LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglyceride concentrations may be the result of mutations in genes encoding the proteins involved in lipid metabolism (4, 5, 8-11). Plasma levels of LDL-C, the main cholesterol-carrying lipoprotein in humans, are determined by the relative rates of LDL production and clearance. Prior to 2003, mutations in the genes coding the LDL receptor *(LDLR)* and apolipoprotein (ApoB) ApoB-100 *(APOB)* had been identified as causes of primary dyslipidemia. Mutations in both of these genes impair LDLR-mediated endocytosis in the liver, which is the main pathway for clearing circulating LDL (12). In 2003, two GOF missense mutations (*S127R* and *F216L*) in the gene encoding proprotein convertase subtilisin/kexin type 9 (PCSK9) were identified in families with a clinical phenotype similar to familial hypercholesterolemia (10). PCSK9, a glycoprotein, regulates the plasma level of LDL-C by increasing the degradation of LDLR (12). To date, the most widely recognized role of PCSK9 is its impact on LDLR; however, recent studies have shown that PCSK9 may also have other effects on

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Table 1. *PCSK9 S127R* (rs28942111), *D374Y* (rs137852912), *F216L* (rs28942112), and *R496W* (rs374603772) primer sequences

Mutation	Forward primer	Reverse primer
S127R	5′-TCACCAAGATCCTGCATGTCT- -3′	5′-TTCAGCAATGGGCCTACTAA-3′
D374Y	5′-TTTGGCCGCTGTGTGGA-3′	5′-GGCTGTTAGCATCACGGTG-3′
F216L	5′-GTATCTCCTAGACACCAGCATACAG-3′	5′-GGAGTACAGCTGCAACGCTCT-3′
R496W	5′-CTGCAGGCCTTGAAGTTGC-3′	5′-GAGCAAATGGATTCAGCTCA-3′

lipoprotein metabolism (triglyceride-rich lipoproteins, chylomicrons, and VLDL) (13).

The aim of this study was to determine the prevalence of *F216L* (rs28942112), *R496W* (rs374603772), *S127R* (rs28942111), and *D374Y* (rs137852912) *PCSK9* GOF mutations in patients with primary dyslipidemia and to investigate the relationship between these mutations and primary dyslipidemia and serum lipid profiles.

Methods

Patients

This observational case-control cross-sectional study included 200 consecutive patients (105 females and 95 males) with primary dyslipidemia who were being followed in the Cardiology Lipid Clinic of Ege University Medical School in İzmir, Turkey, as well as 201 healthy individuals (118 females and 83 males) age- and gender-matched to the study group whose routine health checkups were within normal limits. Patients with secondary causes of hypercholesterolemia (such as hypothyroidism, nephrotic syndrome, diabetes, chronic renal failure, and alcohol consumption) were excluded. Clinical and laboratory data were obtained from the lipid clinic patient files. Family and personal medical histories, such as regarding CV disease, hypercholesterolemia, ischemic stroke, and diabetes mellitus, were obtained. CV risk factors include diabetes mellitus, hypertension, body mass index, and smoking. For the LDL-C, triglycerides, total cholesterol, and HDL-C levels, the maximal values measured before treatment were recorded. The diagnosis of primer dyslipidemia was based on the presence of total cholesterol levels of >200 mg/dL, triglyceride levels of >200 mg/dL, and/or LDL-C levels of >130. The presence of diabetes mellitus was defined as either fasting blood glucose levels of >126 mg/dL or receiving anti-diabetic agent. CVD was identified as the presence of coronary artery disease, aortic aneurysms, dissections or aortic atherosclerotic diseases, ischemic stroke, or peripheral arterial disease; ischemic stroke was defined as a proven cerebral infarct on computed tomography. Therefore, the study population comprised 401 subjects (200 patients with primary dyslipidemia and 201 healthy individuals).

The study protocol was approved by the Institutional Clinical Investigation Ethics Committee (2013, 13-11/92), and all participants gave written informed consent for genetic analysis. The study was supported financially by the Institutional Scientific Research Project Committee (14-TIP-072).

Two 3-mL blood samples were collected from each of the participants into separate EDTA tubes. Detailed personal medical history including the presence of CVD and treatments was received from each participant. In addition, fasting levels of LDL-C, HDL-C, triglyceride, total cholesterol, and fasting glucose were recorded.

Genotyping

Genomic DNA extraction was performed from peripheral blood leukocytes using MagNA Pure LC instrument according to the MagNA Pure LC DNA Isolation Kit I procedure (Roche Applied Science, Mannheim, Germany). Specific primers were designed for the detection of the *PCSK9 S127R* (rs28942111; 381 T>A), *D374Y* (rs137852912; 1120 G>A), *F216L* (rs28942112; 646 T>C), and *R496W* (rs374603772; 1486 C>T) gene mutations (TIB MOLBIOL, Berlin, Germany) (Table 1). These specific primers and High-Resolution Melting Master (HRM) kit (Roche Applied Science, Berlin, Germany) were used to identify *PCSK9 S127R*, *D374Y*, *F216L*, and *R496W* mutations. PCR master mix and thermal profiles for these mutations were optimized according to the HRM kit protocol. All experiments were performed in the LightCycler® 480 Instrument II by real-time online PCR (Roche Applied Science). Polymorphic alleles were identified as wild type, heterozygote, and mutant by the specific melting temperatures (Tm) of the resulting amplicons. The genotype and allele frequencies were evaluated using Hardy–Weinberg Equation. Chi-square test p-values were 0.0012 and 0.0203 for *PCSK9 R496W* (rs374603772) and *D374Y* (rs137852912) mutations, respectively.

Statistical analysis

All statistical analyses were performed using SPSS for Windows version 18.0 (SPSS, Chicago, IL, USA). Data are presented as percentages for discrete variables and as mean±standard deviation for continuous variables. The groups were compared using Student's t-test, and discrete variables were compared using chi-square analysis. P-value of <0.05 (two-sided) was considered to be statistically significant.

Results

Table 2 demonstrates the clinical characteristics and fasting lipid and glucose levels of the study groups. As expected, there

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Table 2. Clinical characteristics and fasting lipid and glucose levels of the study groups

	Primary dyslipidemia group n=200 (100%)	Control group n=201 (100%)	*P*-value
Age, years	50.21±13.2	50.21±13.2	-
Gender			
Male	95 (47.5%)	83 (41.3%)	-
Female	105 (52.5%)	118 (58.7%)	-
Total cholesterol, mg/dL, (min-max)	317.92±105.92 (200-892)	176.15±18.89 (111-200)	<0.001
Triglyceride, mg/dL (min-max)	169.66±87.17 (38-618)	109.28±26.13 (60-186)	<0.001
HDL-C, mg/dL (min-max)	50.08±12.93 (25-96)	63.14±8.99 (37-78)	<0.001
LDL-C, mg/dL (min-max)	237.32±96.86 (71-639)	115.26±17.96 (65-162)	<0.001
Fasting glucose, g/dL (min-max)	95.80±14.89 (62-207)	92.49±12.36 (66-186)	0.82
CVD (+)	60 (30%)	-	-
Diabetes mellitus (+)	18 (9%)	6 (3%)	-

CVD - cardiovascular disease, HDL-C - high-density lipoprotein cholesterol, LDL-C - low-density lipoprotein cholesterol

Table 3. Genotype distributions and allele frequencies of *PCSK9 R496W* (rs374603772) and *D374Y* (rs137852912) in the study groups

PCSK9 gene mutation	Genotype/haplotype	Patient group n=200 (100%)	Control group n=201 (100%)	*P*-value
PCSK9 R496W (rs374603772)	Wild type (CC)	187 (93.5%)	200 (99.5%)	0.005
	Heterozygous (CT)	12 (6%)	1 (0.5%)	
	Mutant (TT)	1 (0.5%)	0 (0%)	
	C	386	401	
	T	14	1	
PCSK9 D374Y (rs137852912)	Wild type (GG)	186 (93%)	197 (99.5%)	0.048
	Heterozygous (GA)	13 (6.5%)	4 (2%)	
	Mutant (AA)	1 (0.5%)	0 (0%)	
	G	385	398	
	A	15	4	

were significant differences between the groups with regard to the presence of elevated total cholesterol, triglycerides, LDL-C levels, and decreased HDL-C levels.

Table 3 shows the summary of the genetic analysis of the 4 *PCSK9* GOF mutations in the study groups. In both *S127R* (rs28942111) and *F216L* (rs28942112) groups, mutations were not detected. Mutation analysis of the 200 patients with primary dyslipidemia showed that 93.5% were wild type (CC), 6% were heterozygous (CT), and only 0.5% was homozygous (TT); meanwhile, of the 201 control subjects, 99.5% were wild type (CC), 0.5% was heterozygote (CT), and none were homozygous (TT) for *PCSK9 R496W* (rs374603772) mutation. This difference in the genotype of *PCSK9 R496W* (rs374603772) mutations was statistically significant between the patient and control groups (χ^2=10.742; p=0.005). Similarly, there was a significant difference between the patient group and controls for the *D374Y* (rs137852912) mutation rates (χ^2=6.078; p=0.048). Analysis of dyslipidemia group revealed that 93% were wild type (GG), 6.5% were heterozygous (GA), and

Table 4. Triglyceride values of patients with heterozygous for R496W (rs374603772) and D374Y (rs137852912) mutations

Mutation	Genotype	Variables in the equation	95% CI EXP		*P*-value
			Lower	Upper	
R496W	Heterozygous	12.834	1.653	99.66	0.015
D374Y	Heterozygous	3.442	1.103	10.746	0.33

Table 5. Clinical characteristics and fasting lipid and glucose levels of the of the PCSK9 mutation carriers and noncarriers in the primary dyslipidemia group

Clinical data	PCSK9 (+) n=25	PCSK9 (–) n=175	*P*-value
Age, years	52.6±12.9	49.8±13.2	-
Female, n, %	10 (40.0%)	95 (54.3%)	-
Male, n, %	15 (60.0%)	80 (45.7%)	-
CVD, n, %	10 (33.3%)	52 (30.6%)	0.125
Total cholesterol	312.6±92.4	318.67±107.9	0.791
Triglycerides	213.4±110.1	163.4±81.8	0.007
HDL cholesterol	48.5±11.4	50.2±13.1	0.532
LDL cholesterol	228.8±78	238.5±99.3	0.643
Fasting glucose	95.4±13.4	95.8±15.1	0.886

CVD - cardiovascular disease, HDL-C - high-density lipoprotein cholesterol, LDL-C - low-density lipoprotein cholesterol

Table 6. Clinical characteristics and fasting lipid and glucose levels of the PCSK9 mutation carriers and noncarriers in the whole study group

Clinical data	PCSK9 (+) n=30	PCSK9 (–) n=371	*P*-value
Age, years	54.4±13.3	51.1±11.2	-
Female, n, %	13 (43.4%)	210 (56.6%)	-
Male, n, %	17 (56.6%)	161 (43.4%)	-
Total cholesterol	289±99.8	243.45±103.6	0.021
Triglycerides	196.9±107.2	134.7±65.1	0.000
HDL cholesterol	51.6±12.8	57±12.8	0.028
LDL cholesterol	211.8±80	173.2±92.9	0.028
Fasting glucose	94.2±13.1	94.1±19.4	0.986

HDL-C - high-density lipoprotein cholesterol, LDL-C - low-density lipoprotein cholesterol

0.5% was homozygous (AA) (Table 3). However, in the control group, 98% were wild type (GG) and 2% were heterozygote (GT).

Comparison of total cholesterol, triglyceride, HDL-C, and LDL-C levels according to the *PCSK9 R496W* (rs374603772) and *D374Y* (rs137852912) genotypes between patients with primary dyslipidemia and healthy controls showed that the triglyceride values of patients with heterozygous for *R496W* (rs374603772) and *D374Y* (rs137852912) mutations were 12.8-fold and 3.4-fold higher, respectively, than those in healthy controls with normal and mutant genotypes (p=0.015 and p=0.03) (Table 4). In addition, when we compared total cholesterol, triglyceride, HDL-C, and LDL-C levels in *PCSK9 R496W* (rs374603772) and *D374Y* (rs137852912) mutation carriers and noncarriers, our results revealed that mutations carriers had higher triglyceride values than noncarriers (p=0.007) (Table 5). But when we compared the *PCSK9 R496W* (rs374603772) and *D374Y* (rs137852912) mutation carriers and noncarriers in the whole study group (n=401) in terms of lipid parameters, significant increase were observed in total cholesterol (p=0. 021), triglycerides (p=0.0001), HDL-C (p=0.028), LDL-C (p=0.028) levels in mutation carriers (Table 6).

Discussion

According to our findings, the prevalence of heterozygote and mutant genotypes in patients with primary dyslipidemic was 6% and 0.5% for *R496W* (1486 C>T) and 6.5% and 0.5% for *D374Y* (1120 G>A), respectively.

The reported PCSK9 GOF mutation frequencies vary in different populations around the world. A 29.7% prevalence was reported among Lebanese patients with familial hypercholesterolemia; a 5.9% *PCSK9 E32K* prevalence was reported in Japanese patients with heterozygous familial hypercholesterolemia; and 1% and 0.9% *PCSK9* mutation prevalence was reported in Portuguese and French patients with familial hypercholesterolemia, respectively (14-17). Moreover, in 2012, Palacios et al. (17) detected *PCSK9* mutation in only 1 of 7,000 Spanish patients, and a public health screening program in Scotland revealed *PCSK9* mutation in only 1 (0.2%) of 425 patients with hypercholesterolemia (18, 19). In the Netherlands, Sjouke et al. (19) reported no *PCSK9* mutations in a genetic analysis of 104,000 patients with hypercholesterolemia, including 49 patients with homozygous familial hypercholesterolemia (20). Similarly, no *PCSK9* mutations were detected in a study conducted in 109 patients with familial hypercholesterolemia in Russia (21). As seen above, there is growing evidence that *PCSK9* mutations play a role in the development of familial hypercholesterolemia. However, its prevalence in primary dyslipidemias is unknown. To the best of our knowledge, our study is first to show the prevalence of *PCSK9 D374Y* and *R496W* mutations in patients with either high LDL-C and/or high triglycerides. Our results showed a higher preva-

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lence of *PCSK9 D374Y* and *R496W* mutations in our Turkish primary dyslipidemia patient group than in the general population. Similarly, we previously reported a 13.8% prevalence of *PCSK9 D374Y* and/or *R496W* GOF mutations in a Turkish familial hypercholesterolemia cohort and have suggested that frequencies of *PCSK9* GOF mutations could be substantially higher in Turkey than other countries (22).

D374Y mutation was first described in 2004 in a Norwegian familial hypercholesterolemia patient group; it was reported to have a prevalence of 4% and be responsible for autosomal dominant hypercholesterolemia (23). In a family genetic study conducted by Timms et al. (23) in Utah, USA, on patients with autosomal dominant hypercholesterolemia, the *D374Y* mutation was also detected in the three other siblings of an individual with *D374Y* mutation (24). In a study designed to investigate the effects of *LDLR, APOB*, and *PCSK9* mutations on the development of CVD in a group of 253 familial hypercholesterolemia patients in the UK, *LDLR* mutation was detected in 236 (57.7%), *APOB* p.Q3500 mutation in 10 (2.4%), and *PCSK9 D374Y* mutations in 7 (1.7%) patients (25). Cameron et al. (25) investigated the effects of the GOF *S127R* and *D374Y* mutations on the autocatalytic activity of PCSK9 in HepG2 cells transfected with LDL in proportion to the amount of LDLR on the cell surface. Their study showed that these mutations did not affect *PCSK9* autocatalytic activity but caused changes in cell-surface LDLR and LDL levels. Cells with the mutant *S127R* and *D374Y* genotype showed 23% reduction in cell-surface LDLR levels and 38% reduction in LDL internalization when compared to cells with wild-type *PCSK9*. The authors reported that the *S127R* and *D374Y* mutations increased the amount of cell-surface LDLR, thereby leading to an increase in the amount of LDL (26). Of the 200 patients with primary dyslipidemia in our study, there were 13 patients with heterozygote (GA) and 1 patient with homozygous (AA) genotype for the *D374Y* mutation (χ^2=6.078; p=0.048). The frequency of the A allele was higher in the patient group than in the control group. Comparison of our results with previous studies suggests that the *PCSK9 D374Y* (rs137852912) mutation has a role in disease development in Turkish patients with primary dyslipidemia.

Pisciotta et al. (26) compared heterozygous patients with familial hypercholesterolemia with a control group and detected *R496W* mutation in only one 35-year-old patient who had a serum LDL level of 518 mg/dL. When plasma LDL-C levels in the proband's mother, who was also heterozygous for *R496W* mutation, were compared with those of other heterozygous familial hypercholesterolemia patients, they reported that the LDL-C levels were similar and that this rarely observed mutation may have pathogenic effect. The authors also reported that *PCSK9 R496W* gene mutation had a negative impact on clinical phenotype in patients with homozygous or heterozygous *LDLR E228K* mutation genotype (27). Of the primary dyslipidemia patients in our study, 12 were heterozygous (CT) and 1 was homozygous (TT) for *R496W* mutation. We also found that heterozygous primary dyslipidemia patients had comparable LDL-C levels and that mutant genotype carriers had a higher LDL-C value than the heterozygous patients. These findings suggest that the CT and TT genotypes of the *PCSK9 R496W* gene mutation can be used as biomarkers for primary dyslipidemia.

PCSK9 mutations were first described by Abifadel et al. (13) in patients with autosomal dominant hypercholesterolemia. They found that the *S127R* mutation was associated with disease progression in a study of autosomal dominant hypercholesterolemia patients from two French families with different ethnic backgrounds. Their analysis of a 49-year-old patient with total cholesterol of 441 mg/dL and LDL-C of 356 mg/dL, who died of a heart attack, and the patient's family revealed *F216L* mutation in the proband and the proband's two children (13). Other studies regarding *S127F* and *F216L* mutations in familial hypercholesterolemia have reported a prevalence of 1.4% in New Zealand and Southern Africa and 2.6% in France (10, 28). In an animal model study, it was also shown that the *PCSK9 S127R* and *F216R* mutant genotypes caused overexpression in the mouse liver, resulting in a dramatic decrease in hepatic LDLR levels because of post transcriptional mechanisms, thereby leading to hypercholesterolemia. In addition, it was determined that overexpression of wild-type *PCSK9* in mice caused plasma levels of total cholesterol to double and non-HDL-C levels to increase 5 fold (29). In an in vivo study evaluating the effects of the *S127R*, *F216L*, and *R218S* mutant genotypes on the PCSK9-dependent LDLR mechanism, Nassoury et al. (29) demonstrated that LDLR was a dominant partner in regulating the cellular trafficking of PCSK9 and that LDLR deficiency resulted in the localization of PCSK9 in the endoplasmic reticulum (ER). They reported that in cells expressing LDLR, PCSK9 was localized with *LDLR* in post-ER compartments. Furthermore, when human wild-type and mutant *S127R*, *F216L*, and *R218S* genotypes were compared in human hepatic HuH7 cells, mutant genotypes were associated with hypercholesterolemia, and PCSK9 was arrested in the endosome/lysosomes and unable to reach the cell surface. Therefore, they demonstrated that the distribution of PCSK9 on the cell surface and endosome is necessary for PCSK9 to completely regulate LDLR degradation and that this retention in the ER causes a reduction in PCSK9 maturation and inhibits *LDLR* degradation. For this reason, they emphasized the causative role of *S127R*, *F216L*, and *R218S PCSK9* mutations in hypercholesterolemia (30). These studies are the extent of the research conducted to date regarding *PCSK9 S127R* and *F216R* mutations. In our study, all individuals in the primary dyslipidemic patient group and the control group carried the wild-type genotype for *PCSK9 S127R* and *F216R* mutations. These data show that the *S127R* and *F216R PCSK9* mutations do not constitute a risk factor for disease development in Turkish patients with primary dyslipidemia. We believe that the discrepancies in the results obtained may have been caused by the selected patient group, genetic heterogeneity, and ethnic differences.

The most widely recognized role of PCSK9 to date has been its impact on LDLR, but recent studies suggest that PCSK9 may

have other effects on lipoprotein metabolism (triglyceride-rich lipoproteins, chylomicrons, and VLDL) as well. Recently, an important study evaluating the relationships between plasma PCSK9 and the VLDL and LDL subfractions showed that plasma PCSK9 was strongly associated with IDL, a triglyceride-rich subfraction that represents VLDL remnants. This finding indicates that PCSK9 exerts its effect on plasma triglycerides by modulating VLDL and VLDL remnant metabolism (31). As further evidence of the link between PCSK9 and triglycerides, it was reported that members of four British families who had familial hypercholesterolemia and carried GOF *D374Y PCSK9* mutations had triglyceride levels that were higher than that in a control group but still within reference ranges (32). This suggests that PCSK9 perturbation may affect triglyceride metabolism (12). In the present study, we also found that triglyceride levels increased by 12.8 fold (p=0.015) and 3.4 fold (p=0.03) in patients with heterozygous *R496W* (1486 C>T) and *D374Y* (1120 G>A) mutations, respectively. Because the studies conducted to date have yielded controversial results, the effect of PCSK9 on triglyceride metabolism is still not fully understood. However, Stein et al. (32) reported in 2014 that 1,359 patients receiving PCSK9 inhibitor treatment showed a significant decrease in triglyceride levels at the end of treatment, regardless of *PCSK9* mutation analysis. This finding illustrates a general connection between PCSK9 and triglyceride metabolism and suggests the possibility of a new therapy for hypertriglyceridemia (32). In our study, we also evaluated the whole study group (n=401) for lipid parameters according to mutation carriers and noncarriers; we found that mutation carriers had higher total cholesterol (p=0. 021), triglycerides (p=0.0001), HDL-C (p=0.028), LDL-C (p=0.028) levels than noncarriers. These results indicate that GOF *R496W* (rs374603772) and *D374Y PCSK9* mutations could have a significant impact on lipid metabolism in general population. However, there are different regions in Turkey, so there may be dominance of different/same PCSK9 mutations in other regions of our country. To show the reflection of *R496W* (rs374603772) and *D374Y* (rs137852912) *PCSK9* mutations in Turkish population, more comprehensive studies need to be conducted from other regions of Turkey.

There is a much lower rate of *PCSK9* mutation in autosomal dominant hypercholesterolemia than that of *LDLR* and *APOB* mutations. However, probands who are heterozygous for both *LDLR* and *PCSK9* mutations have 50% higher plasma LDL levels than relatives with only one mutation (12). *PCSK9* gene constitutes the basis of a new mechanism contributing to dyslipidemia, and determining the effects of the PCSK9 substrate and *PCSK9* gene mutations on function is important in terms of early diagnosis and treatment. A better understanding of the physiology and genetics of lipoprotein metabolism should facilitate earlier diagnosis and development of effective therapeutic interventions for patients with lipid disorders. As research aimed at using antibodies against PCSK9 in the treatment of lipid disorders gains momentum, it is anticipated that these agents will soon become available for clinical use.

Study limitations

The first limitation of the study is the lack of whole-gene sequencing analysis for *PCSK9* gene. The second limitation is homogeneity of the study population that was enrolled from a single experienced lipid specialized center from Aegean region in Turkey, so the results do not reflect the whole Turkish population.

Conclusion

The present study demonstrates the association between the *R496W* (rs374603772) and *D374Y* (rs137852912) *PCSK9* mutations and primary dyslipidemia in Aegeon region. To date, more than 30 GOF mutations have been identified in the *PCSK9* gene. We believe that this study will also lead to other genomic research in this area.

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