

LDLR C1725T Gene Polymorphism Frequency in Type 2 Diabetes Mellitus Patients With Dyslipidemia

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Abstract

Background: Dyslipidemia has a substantial role in the development of cardiovascular diseases in patients with type 2 diabetes mellitus (T2DM). Determining the genetic profile of T2DM patients with dyslipidemia is important in order to reduce the risk of microvascular and macrovascular complications. Low-density lipoprotein receptor (LDLR) plays a critical role in plasma lipoprotein hemostasis. LDLR mutations/polymorphisms cause changes at the lipoprotein level. The objective of this study is to determine the frequency of LDLR (rs179989) polymorphisms in Turkish T2DM patients with dyslipidemia.

Methods: The study group consisted of 217 T2DM patients with dyslipidemia including 28 cases with myocardial infarction and 212 healthy controls. Genomic DNA was isolated from venous blood samples and genotype analysis was carried out on the LightCycler[®] 480 instrument. The χ^2 test was used to compare genotype distributions.

Results: There were no significant differences in the frequency or allelic distribution of the LDLR C1725T (rs1799898) genotype between the type 2 diabetic dyslipidemia patients and the control group ($P > 0.05$).

Conclusion: LDLR C1725T polymorphism was not associated with lipid parameters, and dyslipidemia in T2DM patients.

Keywords: Diabetes mellitus; Dyslipidemia; Lipid metabolism; LDLR; C1725T

Introduction

Diabetes mellitus (DM) occurs due to impairments in insulin secretion and/or function and is characterized by increased blood glucose levels (hyperglycemia) [1]. The prevalence of DM is steadily rising, leading to subsequent increases in many diabetes-related comorbidities [2]. Cardiovascular diseases directly comprise 80% of the increasing mortality in diabetic individuals, and it has been reported that type 2 diabetic individuals have a 2 - 4 times higher risk of developing cardiovascular disease compared to healthy individuals [3-6]. Due to the high morbidity and mortality in these patients, the prevention of cardiovascular complications has become a primary objective in the management of diabetes [7]. In addition to insulin resistance, other cardiovascular risk factors include dyslipidemia, hypertension, susceptibility to coagulation, obesity, hyperinsulinemia and inflammation [8, 9]. Dyslipidemia, one of the most important risk factors, includes various pathologic conditions involving abnormal concentration, composition and distribution of lipids. Recent studies have shown that proteins rich in triglycerides such as chylomicrons and very low-density lipoproteins (VLDL) are significant risk factors for coronary artery disease (CAD), demonstrating that dyslipidemia is an important factor in the pathogenesis of cardiovascular disease in patients with DM. The most common phenotypic characteristics of dyslipidemia in type 2 diabetes are high plasma triglyceride concentration, low high-density lipoprotein cholesterol (HDL-C) concentration and elevated low-density lipoprotein cholesterol (LDL-C) [8].

Low-density lipoprotein receptors (LDLRs) are cell-surface receptors that regulate cellular LDL-C particle intake by receptor-mediated endocytosis and play an important role in plasma lipoprotein hemostasis [10]. LDLR proteins are coded by the LDLR gene consisting of 18 exons of 45 kb in length located on chromosome 19p13. Studies performed to date have identified more than 800 large deletions, small deletions, insertions, point mutations, splice site mutations and polymorphisms of LDLR, and it has been determined that these genetic variations alter individuals' lipoprotein levels [11]. It has also been shown that some of these genetic changes increase the risk of dyslipidemia and cardiovascular disease by increasing levels of total cholesterol and LDL-C in some individuals, while other genetic alterations seem to have a protective effect against cardiovascular disease [12, 13].

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Table 1. Characteristics and the Biochemical Parameters of Diabetic Dyslipidemia Patients and Control Group

	Diabetic dyslipidemia (mean ± SD) (n = 217)	Control (mean ± SD) (n = 212)	P-value
Gender (female/male)	133/84	122/90	NS
Age (years)	53.2 ± 9.8	52.8 ± 9.2	NS
Fasting glucose (mg/dL)	161.9 ± 72.5	87.7 ± 11.6	< 0.001
Total cholesterol (mg/dL)	219.3 ± 61.8	178.2 ± 20.7	< 0.001
Triglycerides (mg/dL)	231.6 ± 255.8	109.5 ± 25.9	< 0.001
HDL cholesterol (mg/dL)	48.5 ± 13.6	63 ± 8.9	< 0.001
LDL cholesterol (mg/dL)	131.6 ± 42.3	117 ± 17.9	
Lipid-lowering therapy (%)	68	-	
Oral antidiabetic therapy (%)	20	-	
Insulin therapy (%)	29	-	

HDL: high-density lipoproteins; LDL: low-density lipoproteins.

In this study, we aimed to investigate the frequency of the LDLR (rs1799898) polymorphism in type 2 diabetes mellitus (T2DM) patients with dyslipidemia and evaluate its association with dyslipidemia.

Materials and Methods

The study group included a total of 217 patients (133 females and 84 males) diagnosed with type 2 diabetic dyslipidemia in the Endocrinology Outpatient Clinic of Ege University Faculty of Medicine, Department of Internal Diseases between January 2009 and September 2011. The control group consisted of 212 healthy individuals (122 females and 90 males) age- and gender-matched to the study group. Type 2 diabetes diagnosis was based on a fasting blood glucose (FBG) level > 126 mg/dL and/or postprandial glucose level > 200 mg/dL and HbA1c level > 6.5%. Subjects with triglyceride levels > 200 mg/dL and/or HDL-C levels < 45 mg/dL were diagnosed with diabetic dyslipidemia. Subjects in the control group did not meet any of these criteria. The study protocol was approved by the Research Ethics Committee of Ege University Faculty of Medicine. Each patient provided a detailed medical history. Biochemical analyses, such as HbA1c, total cholesterol, triglyceride, HDL-C, LDL-C and FBG, were performed with an Olympus AU 2700 chemistry analyzer (Toshiba, Tokyo, Japan).

Blood specimens were collected from each subject into tubes containing ethylenediaminetetraacetic acid (EDTA). Genomic DNA was isolated from peripheral leukocytes of the subjects using MagNA Pure LC DNA Isolation Kit I by MagNA Pure LC DNA isolation instrument (Roche Applied Science). LDLR LightMix® Kit (TIB MOLBIOL) was used to analyze the LDLR C1725T polymorphism. PCR master mix and conditions for LDLR detection were as follows: 10.4 µL H₂O, 1.6 µL 25 mM MgCl₂, 2 µL reagent mix (parameter-specific reagents containing primers and simple probe) (TIB MOLBIOL), 1 µL LightCycler FastStart DNA Master Hybridization Probes (Roche Applied Sciences), 5 µL genomic DNA; denaturation (95 °C for 10 s), 45 cycles amplification (95 °C

for 10 s; 60 °C for 10 s; 72 °C for 15 s), melting analysis (95 °C for 30 s, 40 °C for 2 min, and 75 °C 0 s) and cooling step at 40 °C for 30 s. All experiments were carried out on the Light-Cycler® 480 Instrument (Roche Applied Science, Mannheim, Germany). Polymorphic alleles were identified by the specific melting temperatures of the resulting amplicons, as individuals with two copies of the C allele (CC) showed a single melting peak at 64 °C, individuals with two copies of the T allele (TT) showed a single melting peak at 57 °C, and individuals with both alleles (CT) showed two melting peaks at 57 and 64 °C in the melting curve analysis.

The biochemical data of type 2 diabetic dyslipidemia patients and healthy control group were compared in terms of the genotype distribution of the LDLR C1725T polymorphism.

Statistical analysis

All statistical analyses were performed using SPSS for Windows (Version 18.0, SPSS, Chicago, IL, USA). The data were presented as percentages for discrete variables and as mean ± standard deviation (SD) for continuous variables. A P value of < 0.05 (two-sided) was considered statistically significant. Comparisons between groups were made by *t*-test, and discrete variables were compared by Chi-square analysis.

Results

The study population comprised 217 unrelated Turkish type 2 diabetic dyslipidemia cases (133 females and 84 males), including 28 subjects (13 female, 15 males) with myocardial infarction (MI) and 212 controls (122 females and 90 males). The comparison of clinical characteristics and biochemical parameters of the groups is shown in Table 1. FBG, total cholesterol, triglycerides, and LDL-C levels were significantly higher in the diabetic dyslipidemia group than in the control group; whereas HDL-C levels were significantly lower (*P* < 0.001). Patients in the diabetic dyslipidemia group were treated with oral antidiabetics (*n* = 68), insulin (*n* = 20) and lipid-lowering

Table 2. Genotype Distribution and Allele Frequency of LDLR C1725T (rs1799898) Gene Polymorphisms in Diabetic Dyslipidemia Patients and the Control Group

Gene/SNP	Genotypes haplotype	Patients, n (%)	Control, n (%)	P-value
LDLR C1725T (rs1799898)	CC	143 (66.2)	142 (67.0)	0.256
	CT	70 (32.4)	62 (29.2)	
	TT	3 (1.4)	8 (3.8)	
	C	356	346	
	T	76	78	

agents (n = 29) (Table 1).

Wild-type (CC), heterozygous (CT) and polymorphic (TT) genotype distributions of the LDLR (rs1799898) polymorphism were found to be 66.2%, 32.4%, and 1.4% in the type 2 diabetic dyslipidemia group and 67%, 29.2%, and 3.8% in the control group, respectively. There was no significant difference between the groups' distributions of LDLR (rs1799898) polymorphisms (Table 2).

Moreover, we also compared genotype distribution of LDLR (rs1799898) gene polymorphism with type 2 diabetic dyslipidemia patients and type 2 diabetic dyslipidemia patients with MI. For the LDLR C1725T polymorphism, distribution of the CC, CT, and TT genotypes was found to be 66%, 32.4%, and 1.6% and 67.9%, 32.1%, and 0% in the type 2 diabetic dyslipidemia patients and type 2 diabetic dyslipidemia patients with MI groups, respectively (Table 3). However, we did not find significant association for frequencies of C1725T genotypes.

A comparison of biochemical parameters such as HbA1c, FBG, total cholesterol, triglycerides, HDL-C, and LDL-C levels between diabetic dyslipidemia patients and control group according to LDLR (rs1799898) genotype revealed no significant correlations between lipid parameters, plasma glucose, HbA1c levels and genotype distribution ($P > 0.005$).

Discussion

Modern changes in lifestyle and nutrition habits are increasing the incidence of T2DM considerably, together with the influence of hereditary and environmental factors. The relation between DM and serum lipid profiles has been discussed for the last decade [14]. From studies of T2DM patients, it is known that diabetic dyslipidemia occurs not only as a disruption of lipoprotein metabolism resulting from changes in the quantitative and qualitative characteristics of lipoproteins, but may also develop due to genetic and environmental factors. Therefore, determining the genetic profile of diabetic patients

with dyslipidemia is considered important in terms of reducing the risk of possible microvascular and macrovascular complications. To date, no previous studies have reported the effects of LDLR (rs1799898) polymorphism in diabetic dyslipidemia patients. This study is the first comprehensive study investigating the frequency of LDLR (rs1799898) polymorphisms in Turkish type 2 diabetic dyslipidemia patients. According to our analysis, the wild-type, heterozygote, and polymorphic genotype rates are 66.2%, 32.4%, and 1.4% in type 2 diabetic dyslipidemia patients and 67%, 29.2%, and 3.8% in controls, respectively.

LDLR is a membrane glycoprotein which regulates the uptake and degradation of cholesterol-rich lipoproteins by receptor-mediated endocytosis [10, 12]. LDLR mutations/polymorphisms change individuals' LDLR protein structures and functions and lead to the formation of different clinical phenotypes [11, 15, 16]. Several studies have investigated whether common SNPs in LDLR such as rs12983082, rs2738446, rs1799898, rs9789302, rs5925, and rs688 contribute to individual variations in serum lipid concentrations. Tejedor et al investigated the effects of LDLR (rs1799898) polymorphisms in familial hypercholesterolemia patients and did not detect any remarkable phenotypic effects on disease development, as in our study [17]. Jamaldini et al investigated the effects of LDLR C1725T polymorphisms on the development of CAD and reported that CC, CT, and TT genotype rates were 79.41%, 17.65%, and 2.94% in CAD patients and 64.08%, 32.04%, and 3.88% in the control group, respectively. In contrast to the current study, Jamaldini supported that the CT or TT genotypes of C1725T (rs1799898) SNP may have a protective effect against CAD [13]. In our study, only 28 type 2 diabetic dyslipidemia cases had MI. Therefore, the number of the patients with MI is too small to determine the association between LDLR C1725T gene polymorphism and cardiovascular disease in our study.

In genetically heterogeneous populations, obtaining contradictory results can be attributed to regulation by gene-gene and gene-environment interactions and/or diversity of patients' characteristics. Type 2 diabetic dyslipidemia is a complex dis-

Table 3. Genotype Distribution of LDLR C1725T (rs1799898) Gene Polymorphisms in Type 2 Diabetic Dyslipidemia Patients and Type 2 Diabetic Dyslipidemia Patients With MI

Gene/SNP	Genotypes haplotype	Patients, n (%)	Patients with MI, n (%)	P-value
LDLR C1725T (rs1799898)	CC	124 (66.0)	19 (67.9)	> 0.05
	CT	61 (32.4)	9 (32.1)	
	TT	3 (1.6)	0 (0)	

ease influenced by genetic and environmental factors. Therefore, it is difficult to definitively identify pathogenetic factors. Further studies with larger patient numbers in different/same populations are needed. In future studies, other LDLR polymorphisms/mutations should be investigated in order to determine their effects on individual variations in lipoprotein levels and disease development.

Financial Disclosure

No competing financial interests exist.

Conflicts of Interest

There are no conflicts of interest.

Author Contributions

Concept: ZE, EH, and ATV. Design: ATV and ZE. Supervision: ZE and ATV. Materials: EH and EV. Data collection and/or processing: ATV, EH, EV, and MK. Analysis and/or interpretation: ATV and MK. Literature search: ZE and ATV. Writing: ZE and ATV. Critical review: AZ, ZE, VBC, EV, and ASK.

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