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Sequence Variant in the LPIN1 gene in Patients with Metabolic Syndrome

[Metabolik Sendrom Hastalarında LPIN1 Geni Dizi Varyantı]

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ABSTRACT

Objective: Metabolic syndrome (MetS) is a complex disease characterized by insulin resistance, abdominal obesity, hyperglycemia, hypertension, hypertriglyceridemia and low HDL-cholesterol level. The aim of the study was to evaluate the sequence variations in the LPIN1 gene in MetS. This gene codes lipin-1 protein which functions as Mg-dependent phosphatidic phosphatase enzyme and transcriptional coactivator.

Material and Methods: The study groups included 73 MetS (19 M/54 F) and 56 non-MetS (16 M/ 40 F). Sequence variation in exons 2, 4, 5 and 14 of the LPIN1 gene were investigated by DNA sequencing method.

Results: c.696 G>C variant (p.S232S) in exon 5 was observed in only one women with MetS. But this variation is not important because of coding same amino acid.

Conclusion: Any important sequence variant was not detected in exons 2, 4, 5 and 14 in the LPIN1 gene in MetS.

Key Words: metabolic syndrome, LPIN1, lipin-1

Conflict of Interest: Authors have no conflict of interest.

ÖZET

Amaç: Metabolik sendrom (MetS) insülin resistansı, abdominal obezite, hiperglisemi, hipertansiyon, hipertrigliseridemi ve düşük HDL kolesterol seviyeleri ile karakterize kompleks bir hastalıktır. Bu çalışmanın amacı, MetS'de LPIN1 geni dizi değişikliklerini değerlendirmekti. Bu gen, Mg-bağımlı fosfatidik asit fosfataz enzimi ve transkripsiyon koaktivatörü olarak fonksiyon gören lipin 1 proteinini kodlamaktadır.

Gereç ve Yöntemler: Çalışma grubu, 73 MetS (19 E/54 K) olan ve 56 MetS olmayan (16 E/ 40 K) kişiden oluşturuldu. LPIN1 geninde ekzon 2, 4, 5 ve 14'de dizi varyasyonu, DNA dizi analizi yöntemi ile araştırıldı.

Bulgular: Sadece bir bayan hastada ekzon 5'de c.696 G>C (p. S232S) değişimi görüldü. Ancak aynı amino aside denk geldiği için bu değişim önemli değildir.

Sonuçlar: MetS da LPIN1 ekzon 2, 4, 5 ve 14'de önemli bir dizi değişimi saptanmadı.

Anahtar Kelimeler: metabolik sendrom, LPIN1, lipin-1

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

The metabolic syndrome (syndrome X) (MetS) is a constellation of clinical and biochemical abnormalities characterized by dyslipidemia [hypertriglyceridemia and decreased HDL-C (high density lipoprotein cholesterol)], hypertension, impaired glucose tolerance and central obesity [1, 2]. Other metabolic abnormalities associated with MetS are abnormal weight distribution, inflammation, microalbuminuria, hyperuricemia, nephropathy, low tissue plasminogen activator and abnormalities of fibrinolysis and of coagulation [3, 4]. Thereby, this syndrome has been identified as a multiplex risk factor for cardiovascular diseases [5].

Lipin-1 is Mg^{2+}-dependent "type 1" phosphatidic acid phosphatase (PAP1; 3-sn-phosphatidic acid phoshohydrolyse, EC 3.1.3.4) that dephosphorylate phosphatidic acid (PA) to generate diacylglycerol which is the immediate precursor of triacylglycerol, phosphatidylcholine, and phosphatidylethanolamine [6, 7]. Moreover, lipin-1 acts as a transcriptional coactivator/ corepressor for metabolic nuclear receptors regulating fatty acid metabolism in liver such as peroxisome proliferator-activated receptor α (PPARα) and PPARγ coactivator 1α (PGC-1α) [6, 8]. In the presence of elevated fatty acid levels within the cell, lipin proteins translocate from the cytosol to the endoplasmic reticulum membrane to metabolize PA [9]. Lipin-1 is abundantly expressed in adipose tissue, skeletal muscle and liver, to regulate lipid metabolism and glucose homeostasis [10, 11]. Therefore, the lack of lipin-1 prevents differentiation of human adipocyte precursor cells *in vitro* and causes lipodystrophy syndrome *in vivo*. On the other hand, lipin-1 polymorphisms have been associated with numerous metabolic traits, including insulin and glucose levels, resting metabolic rate and systolic blood pressure [12]. The increased lipin-1 expression in adipose tissue is associated with enhanced insulin sensitivity [13]. In addition, lipin-1 acts as a key integrator of hormonal signals to the liver [14]. Therefore, fasting, diabetes, high fat diet, chronic alcoholism, statin and glucocorticoid intake lead to increase of hepatic lipin-1 expression [15]. In liver, lipin-1 protein also involve in formation and secretion of very low density lipoprotein [14, 16].

Human lipin-1 protein is encoded by the *LPIN1* gene (MIM605518, Entrez Gene ID 23175) which is expressed in three isoforms including lipin-1α (890 amino acids), lipin1β (926 amino acids) and lipin-1γ (916 amino acids) [6, 14]. *LPIN1* gene polymorphisms were associated with abnormal insulin, high-, low- density lipoprotein and total cholesterol levels, suggesting its role in lipid metabolism and pathogenesis of type 2 diabetes and MetS [17].

The aim of the study was to analyze the *LPIN1* gene sequence variants and their relationship with MetS diagnostic criteria. Among 20 exons of LPIN1 gene, exons 2, 4, 5 and 14 were particularly selected due to reported polymorphisms in these exons [18].

Materials and Methods

Study Population

The study was carried out in the Departments of Medical Biochemistry and Internal Medicine of the Faculty of Medicine of Karadeniz Technical University. All the participants gave informed consents and the study protocol was approved by the Local Ethical Board of the Faculty of Medicine (No.2006/25).

The subjects were considered to have MetS if they had any three or more of the following criteria of National Cholesterol Education Program (NCEP)/Adult Treatment Panel (ATP) III [19];

Abdominal obesity: Waist circumference >102 cm in men and >88 cm in women.

Hypertriglyceridemia: Serum triglycerides (TG) level ≥ 150 mg/dL (1.69 mmol/L).

Low HDL-C: <40 mg/dL (1.04 mmol/L) in men and <50 mg/dL (1.29 mmol/L) in women.

High blood pressure: Systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg or on treatment for hypertension.

High fasting blood glucose: Serum glucose level ≥ 110 mg/dL (6.1 mmol/L) or on treatment for diabetes.

Waist circumference (WC) was measured in duplicate at the narrowest horizontal point between costal margin and iliac crests at the end of normal expiration to the nearest 0.1 cm. SBP and DBP were measured thrice in sitting position after 15 min rest, and the mean value was taken for all cases. Participants were advised to avoid caffeinated beverages and exercise for at least 30 min before.

The study groups included 73 patients with MetS (19 men and 54 women) and 45 non-MetS healthy volunteers (16 men and 40 women). The patient group has the criteria in the following ratio: Abdominal obesity 73/73, hypertriglyceridemia 71/73, low HDL-C 64/73, hypertension 67/73 and high fasting glucose 28/73. Among MetS patients, 14 of them had all 5 criteria and the others had 4 criteria.

Determinations of the Biochemical Parameters

Blood samples were collected after 10–12 h fasting and the sera were separated by centrifugation. Aliquots of sera were then taken and stored at −80 °C until the tests were performed. Serum TG levels were measured by using a glycerol oxidase enzymatic method. HDL-cholesterol by a cholesterol oxidase enzymatic method in supernatant after precipitation with phosphotungstic acid–$MgCl_2$; fasting serum glucose was measured by using an enzymatic (glucose oxidase) colorimetric

method. All above measurements were performed with an autoanalyzer (Roche, Modular, Switzerland).

DNA Sequencing of Exons 2, 4, 5 and 14 of the LPIN1 Gene

DNA was isolated from whole blood samples by using Invisorb Spin Blood Mini Kit (Cat No: 1031100200; Berlin, Germany). Polymerase chain reaction (PCR) steps (with little modification) and primer sequences of exons were chosen according to Cao and Hegele [18]. Amplification conditions for the *LPIN1* exons were as follows: 94°C for 5 min, followed by 30 cycles of 60 s each at 94°C, 60°C, and 72°C and ending with a single 10-min extension step at 72°C. Primer sequences were as follows:

Exon 2 F: 5'- CTT GGA TTA ATT GTG TGT CTG TGT G -3'

R: 5'- TTT CAA TTA GTC ACT GTC AGT TCC G- 3'

Exon 4 F: 5'- CAA GGC CCT GCT TCT TAT ACC T - 3'

R : 5'- AGG ATT TAT GGG GGA AAA GTTC - 3'

Exon 5 F: 5'- CAA AGC ACT TAG AGC TAA TCA AGA AA - 3'

R: 5' – TGA CTT CTA AGC CTC TGC ACT G - 3'

Exon 14 F: 5' - ATT TTT CAT GGC TAC CCA GAT G - 3'

R: 5' - AAA CCT CAT GTG CTC ACA ACA C - 3'

The PCR products were purified by using purification kit (Invisorb Spin PCRapid Kiti-Invitek Lot no: BC070014). BigDye Cycle Sequencing v3.1 Kit (Applied Biosystems, California, USA) was used for cycle sequencing reactions. Cycle sequencing was performed for 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. The products were repurified using Sephadex G-50 (Sigma-Aldrich, Germany). The final products were used for DNA sequencing analysis by ABI PRISM 3130 Genetic Analyzer. BioEdit (BioEdit, Carlsbad, CA) software mutation charts were used for the sequencing analysis.

Statistical Analysis

Data were expressed as the means and standard deviations (S.D.). The distributions of the variables in study groups were assessed by Kolmogorov–Simirnov test. Statistical differences between data of MetS and non-MetS groups were determined according to Student's *t*-test (parametric). *P*-values less than 0.05 were considered to be statistically significant.

Results

The values of anthropometric and biochemical parameters of MetS and non-MetS individuals were shown in Table 1. The values of WC, SBP and DBP and the levels of glucose and TG were significantly higher but HDL-C was lower in MetS group than in non-MetS group.

As a result of DNA sequencing analysis of the *LPIN1* exons, it was observed that only one patient with 5 criteria had c.696 G>C transition in exon 5 of *LPIN1* with SNP number rs145180224. Since this transition doesn't change the amino acid sequence in protein level, it is a silent variation (p.S232S) (Figure 1). The values of the anthropometric and biochemical parameters belong to this patient were given in Table 2. Any other variant was not obtained in any exons in both MetS and non-MetS group.

Discussion

Lipin-1 protein, a product of the *LPIN1* gene, modulates lipid metabolism and glucose homeostasis [10]. While cytosolic lipin-1 acts as a PAP enzyme converting phosphatidate to diacylglycerol during triglyceride biosynthesis, nucleotic lipin-1 acts as a transcriptional coactivator interacting with PPARα and PGC-1α complex that modulates fatty acid oxidation gene expression [20]. In humans, variations in lipin-1 expression levels and gene polymorphisms are associated with insulin sensitivity, metabolic rate, hypertension, and risk for the metabolic syndrome [21]. In addition, adipose tissue *LPIN1* expression levels were reported to reduce in obesity and the metabolic syndrome [22].

In this study, the sequence of exon 2, 4, 5 and 14 of the *LPIN1* gene were screened by DNA sequencing method in MetS and non-MetS groups. MetS group had significantly higher values of WC, SBP, DBP, glucose and TG but lower values of HDL-C than non-MetS group ($p<0.001$) (Table 1). 14 of the MetS subjects fulfilled all 5 criteria and the others fulfilled criteria. As a result, sequence variation was not detected in the exons of *LPIN1* analyzed in MetS and non-MetS subjects, except c.696 G>C (SNP rs145180224) (p.S232S) variant seen in exon 5 of one MetS patient (Figure 1). The women with c.696 G>C variant in exon 5 in *LPIN1* gene had 5 of the MetS criteria (Table 2). This variant codes same amino acid (silent mutation) in human lipin-1 protein having 890 amino acids.

Even though it was not detected in our study, many single nucleotide polymorphisms (SNPs) in exons 2, 4, 5 and 14 of the *LPIN1* were reported in PubMed [23]. In addition, there are also some studies reporting many variants in *LPIN1* gene. For instance, Cao and Hegele [18] identified a nonsynonymous SNP 2211C>T in exon 14 in the lipodystrophy syndrome and in normal control groups. Fawcett et al. [24] reported S232S (G>C) (as detected in the present study), A353T (G>A), V494M (G>A), R552K (G>A), G582R (G>A) and P610S (C>T) SNPs and found the rare nonsynonymous variants A353T, R552K, and G582R in severely insulin-resistant patients, but did not found any association between *LPIN1* SNPs and fasting insulin. According to Zhang et al. [25], rs16857876 and rs11695610 SNPs within the LPIN1

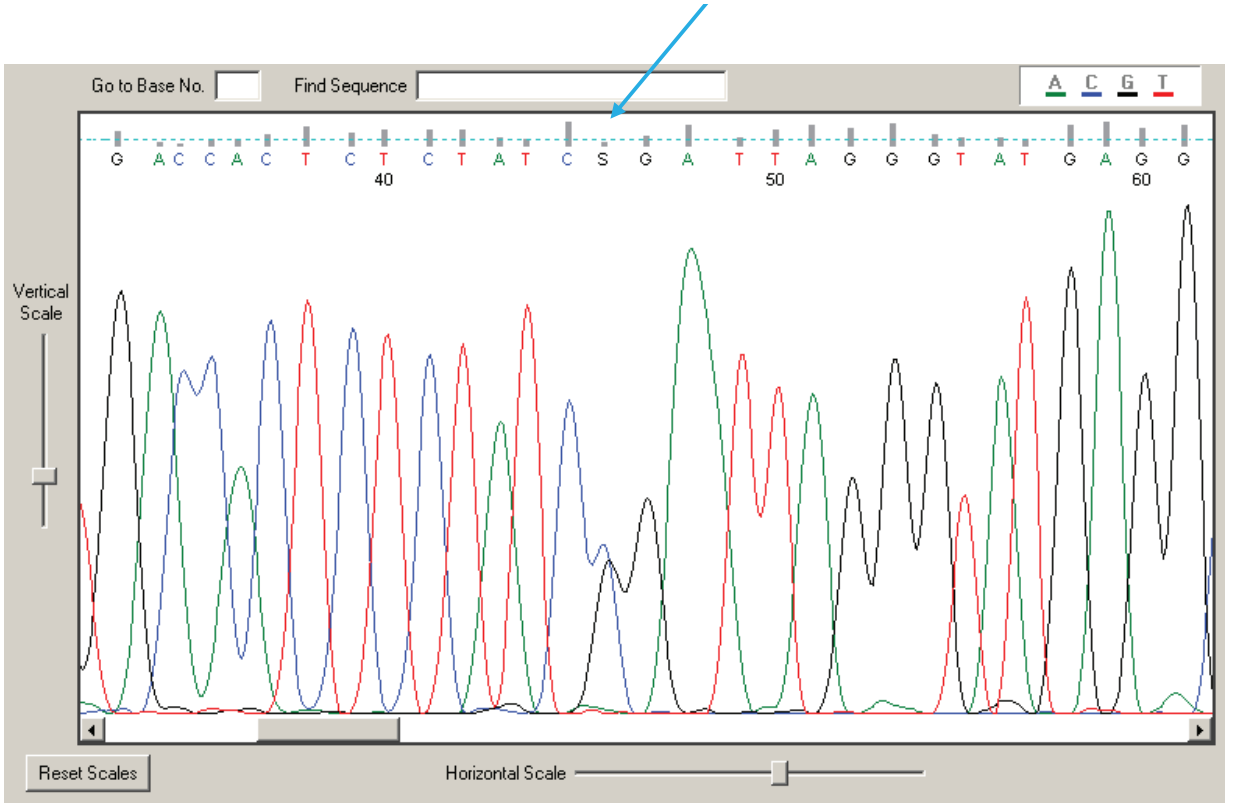

Figure 1. The electrophoregram of the LPIN1 gene exon 5 of MetS patient with c.696 G>C variant, obtained with ABI PRISM 3130 Genetic Analyzer.

Table 1. The values of the anthropometric measurements and the biochemical parameters in MetS and non-MetS groups.

	non-MetS N=56	MetS N=73	*p*
F/M	40/16	54/19	<0.05
Age (years)	33 ± 8	52 ± 15	< 0.001
WC (cm)	78 ± 9	106 ± 11	< 0.001
SBP (mmHg)	113 ± 9	150 ± 28	< 0.001
DBP (mmHg)	73 ± 7	93 ± 16	< 0.001
TG (mg/dL)	68 ± 23	233 ± 96	< 0.001
HDL-C (mg/dL)	66 ± 12	41 ± 7	< 0.001
Glucose (mg/dL)	83 ± 9	120 ± 68	< 0.001

F: Female, M: Men, WC: Waist circumference, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TG: Triglyceride, HDL-C: High density lipoprotein-cholesterol.

region were associated with type-2 diabetes in Chinese population. But in another study with Chinese population, it was reported that the *LPIN1* gene seemed not to be a major susceptibility gene for type-2 diabetes or related metabolic phenotypes [26]. Burgdorf et al. [27] determined that *LPIN1* rs33997857, rs6744682, and rs6708316 did not associate with type-2 diabetes, obesity, or related diseases in the Danish population. Loos et al. [20] explained that *LPIN1* IVS18 + 181C>T variant (rs2716609) showed a strong association with fasting insulin levels and with measures of adiposity and fat distribution. In addition, they suggested that *LPIN1* gene contributes to variation in obesity-related phenotypes, potentially in an age-dependent manner. Suviolahti et al. [28] found the strong negative correlations of lipin expression with glucose and insulin in the patients with

Table 2. The values of the anthropometric measurements and the biochemical parameters of MetS patient with c.696 G>C variant in exon 5 of the *LPIN1* gene.

Age (years)	54
WC (cm)	105
SBP (mmHg)	130
DBP (mmHg)	90
TG (mg/dL)	172
HDL-C (mg/dL)	40
Glucose (mg/dL)	119

dyslipidemia. As seen in the given studies above there are conflicting results about the *LPIN1* gene and insulin resistance.

To the best of our knowledge, there was no study in Turkish population about the lipin-1 protein and the *LPIN1* gene. The reason why the previously reported polymorphisms or mutations of LPIN1 exons were not detected in this current study can be explained by polymorphisms specific to Turkish population of this particular gene. Another reason may be the small number of subjects. In addition, although the *LPIN1* gene has 20 exons, only 4 exons (2, 4, 5 and 14) were screened. The limitations of this present study were the number of the subjects and the limited exons.

In conclusion, no important sequence variation in exons of the *LPIN1* gene was associated with MetS. But the current study suggested that the new study with large number of subjects is necessary to detect the genetic variation of the *LPIN1* in Turkish population.

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Conflict of interest: Authors have no conflict of interest.

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