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Determination of genetic changes of *Rev-erb beta* and *Rev-erb alpha genes* in Type 2 diabetes mellitus by next-generation sequencing

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ABSTRACT

Background: The nuclear receptors Rev-erb alpha and Rev-erb beta are transcription factors that regulate the function of genes in glucose and lipid metabolism, and they also form a link between circadian rhythm and metabolism. We evaluated the variations in *Rev-erb alpha* and *Rev-erb beta* genes together with biochemical parameters as risk factors in type 2 diabetic (T2DM) patients.

Methods: Molecular analyses of *Rev-erb alpha* and *Rev-erb beta* genes were performed on genomic DNA by using next-generation sequencing in 42 T2DM patients (21 *obese* and 21 *non-obese*) and 66 healthy controls.

Results: We found 26 rare mutations in the study groups, including 13 missense mutations, 9 silent mutations, 3 5′UTR variations, and a 3′UTR variation, of which 9 were novel variations (5 missense and 3 silent and 1 5′UTR). Six common variations were also found in the *Rev-erb* genes; *Rev-erb beta* Chr3:24003765 A > G, *Rev-erb beta* rs924403442 (Chr3:24006717) G > T, *Rev-erb alpha* Chr17:38253751 T > C, *Rev-erb alpha* rs72836608 C > A, *Rev-erb alpha* rs2314339 C > T and *Rev-erb alpha* rs2102928 C > T. Of these, Rev-erb beta Chr3:24003765 A > G was a novel missense mutation (p.Q197R), while others were identified as intronic variants.

T2DM patients with *Rev-erb beta* rs924403442 T allele had lower body surface area (BSA) than noncarriers (GG genotype) ($p = 0.039$). *Rev-erb alpha* rs72836608 A allele and *Rev-erb alpha* rs2314339 CC genotype were associated with decreased serum HDL-cholesterol levels in T2DM patients ($p = 0.025$ and $p = 0.027$, respectively). In our study, different effects of Rev-erbs polymorphisms were found according to gender and presence of obesity. *Rev-erb alpha* rs72836608 (C > A) and rs2314339 (C > T) and *Rev-erb alpha* rs2102928 (C > T) were associated with low HDL-C levels in male T2DM patients. In female patients, *Rev-erb alpha* rs2102928 (C > T) was associated with high microalbuminuria and Rev-erb beta rs9244403442 G > T was associated with low HDL and high BSA values. In addition, *Rev-erb alpha* Chr17: 38,253,751 (T > C), rs72836608 (C > A), and rs2314339 (C > T) and *Rev-erb beta* Chr3:24003765 (A > G) were associated with increased serum GGT levels in *obese* T2DM patients. In *non-obese* patients, *Rev-erbs* SNPs had no effect on serum GGT levels.

Conclusion: Our findings indicate that variations in the *Rev-erb alpha* and *Rev-erb beta* genes can affect metabolic changes in T2DM and these effects may vary depending on gender and obesity.

Abbreviations: ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BG, Blood glucose; BMI, Body mass index; CRP, C-Reactive Protein; DBP, Diastolic blood pressure; DM, Diabetes mellitus; fT4, Free thyroxine; GGT, Gamma-Glutamyl Transferase; Hb, Hemoglobin; HC, Hip circumference; Hct, Hematocrit; HbA1c, Glycated hemoglobin; HDL-C, High density lipoprotein cholesterol; LDL-C, Low density lipoprotein cholesterol; SBP, Systolic blood pressure; sT3, Serum triiodothyronine; TC, Total cholesterol; TG, Triglyceride; TSH, Thyroid Stimulating Hormone; VLDL-C, Very low density lipoprotein cholesterol; WC, Waist circumference

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1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the most important health problems in modern society and affects millions of people around the world. Obesity and excessive weight gain play an important role in the development of T2DM along with other risk factors such as genetic predisposition and lack of physical activity (International Diabetes Federation, 2017).

Circadian rhythms are a transcriptional mechanism involving repetitive physiological and behavioral cycles over 24 h generated by the suprachiasmatic nucleus in the anterior hypothalamus (Golombek and Rosenstein, 2010). These rhythms control various biological processes such as the sleep-wake cycle, body temperature, nutrition, glucose homeostasis, hormone secretion, and regulation of the cell cycle. Loss of coordination of these physiological rhythms can have a negative effect on physiological and behavioral functions (Zee et al., 2013). Since the circadian clock is associated with the secretion of a large number of hormones that are critical for maintaining normal physiological processes, the circadian rhythm and metabolism are closely intertwined (Eckel-Mahan and Sassone-Corsi, 2013; Bandin et al., 2015; Ruiz-Lozano et al., 2016). Studies showing that impaired circadian rhythm cause the development of metabolic disorders such as hyperlipidemia, hyperglycemia, and varying insulin sensitivity in animals and humans confirm this link between circadian clock function and metabolism (Rudic et al., 2004; Huang et al., 2011). In fact, circadian disorders have been reported to cause T2DM (Gale et al., 2011; Taguchi et al., 2018). Furthermore, several studies reported the association of circadian rhythm disruption with obesity, cancer or sleep and behavioral disorders (Garaulet et al., 2010; Turek et al., 2005; Vieira et al., 2014a).

Rev-erb alpha and Rev-erb beta are important transcription factors that regulate genes involved in cellular differentiation, inflammation, glucose and lipid metabolism linking circadian rhythm (Burris, 2008; Solt et al., 2012; Sato et al., 2014; Nam et al., 2015; Carter et al., 2017; Cho et al., 2012; Bugge et al., 2012). Rev-erbs (alpha and beta) belonging to the ligand-regulated nuclear receptor superfamily are expressed in many tissues, particularly adipose tissue, skeletal muscle, brain, kidney, and liver (Burris, 2008; Evans, 2013). Unlike many nuclear receptors, Rev-erbs do not contain the activation function-2 region (AF2) (transcription activation domain) in their ligand-binding domains (LBDs), making it impossible for them to interact with a coactivator. Therefore, the Rev-erbs function as repressors of genes containing Rev-erb response elements (RevREs) in the promoter (Woo et al., 2007). However, Rev-erb beta has been shown to activate sterol regulatory element-binding protein-1c (Srebp-1c) in skeletal muscle (Ramakrishnan et al., 2009). Rev-erb alpha and Rev-erb beta directly regulate the circadian rhythm by targeting the circadian clock genes, Bmal1 and Clock, in the brain and peripheral tissues (Preitner et al., 2002; Guillaumond et al., 2005).

In the study of Kang et al., the relationship between *Rev-erb alpha (NR1D1)* gene rs2314339, rs2071427, rs12941497 SNPs and chronotypes classified as M, I and E according to CSM criteria (circadian typologies) and a significant relationship has been reported chronotype category between rs12941497 genotypes which supports the role of *Rev-erb* polymorphisms in circadian rhythm regulation. In another study, *Rev-erb alpha* rs2314339 T allele was found to be associated with bipolar disorder (Kripke et al., 2009; Partonen, 2012; Campos-de-Sousa et al., 2010). Moreover, Campos et al. reported that the rs2314339 T allele was associated with a worse response to lithium therapy in bipolar affective disorder (Campos-de-Sousa et al., 2010). Besides, animal studies also supports the interaction of Rev-erb genes with circadian behavior. Choe et al. reported an impaired circadian behavior in Rev-erb alpha and Rev-erb beta deficient mice (Cho et al., 2012). Bugge et al. showed that the silencing of Rev-erb alpha and Rev-erb beta inhibited circadian gene expression in mouse embryonic fibroblasts (Bugge et al., 2012). Due to their effects in all these processes, Rev-erb genes are known to play a role in the pathogenesis of diseases such as diabetes, obesity, atherosclerosis, and cancer (Yang et al., 2013).

Based on the important roles of the Rev-erb nuclear receptor gene family in the regulation of genes involved in lipid/carbohydrate metabolism, circadian rhythm, and inflammation, we aimed to analyze the relationship of *Rev-erb alpha* and *Rev-erb beta* gene variations with biochemical and clinical parameters in T2DM patients with and without obesity and healthy controls.

2. Materials and Methods

2.1. Patients characteristics

The study was approved by both the Ethics Committee of the Faculty of Medicine and the Research Fund of Istanbul University, and written informed consent was obtained from each participant before the study (Approval Date/ No: 27.3.2015/ 06).

The patient group consisted of 42 cases diagnosed with T2DM followed by the Diabetes Polyclinic of the Division of Endocrinology and Metabolic Diseases, Department of Internal Medicine, Istanbul Faculty of Medicine. The control group consisted of 66 healthy individuals without any metabolic disorders (diabetes mellitus, lipid metabolism disorder, liver failure, renal failure, etc.), familial early age ischemic heart disease and hypertension.

2.2. Genetic analysis

2.2.1. DNA isolation

Genomic DNA was extracted from human leukocyte nuclei isolated from whole blood by DNA extraction kit (MasterPure™ Epicentre DNA Purification Kit, Madison, WI, USA). The quality control of DNA was performed with a Qubit fluorometer (Invitrogen, CA, USA) and 1% agarose gel electrophoresis.

2.2.2. Next generation sequencing for Rev erb genes

The exons and their adjacent regions of ± 100 base pair, and 5′ upstream and 3′ downstream regions of *Rev-erb alpha* and *Rev-erb beta* genes were sequenced in 66 control subjects and 42 T2DM patients. *Rev-erb alpha* and *Rev-erb beta* gene regions were amplified by long polymerase chain reaction (PCR). The PCR products were then purified and quantified with Qubit fluorometer (Invitrogen, CA, USA). The DNA for each sample was adjusted to 2 nmol and pooled to create the DNA library, before initiating the Next Generation Sequencing (NGS) process. Each DNA sample was equalized to 50 ng/μl and examined by NGS (Miseq sequencing system, Illumina, San Diego, CA, USA).

With an average of 221,000 units of 2 × 250 bp readings per sample, an average of 35 Mb of data was obtained per sample. After sequencing, the readings at the target region were filtered from the sequence data obtained. The raw sequence data obtained were cleaved according to the quality scores (Trimmomatic v0.27). The corrected raw sequence data were aligned to the human genome reference sequence (hg19, GRCh37) by using Burrows-Wheeler Aligner (v.0.7.12) (Li and Durbin, 2010). The re-alignments around insertions and deletions (indels) were performed using the Genome Analysis Toolkit v3.3.0 (GATK) IndelRealigner. After combining and aligning, GATK (v3.3.0) was used to filter the repetitive readings to optimize the number of readings and re-calibrate base quality. In the last step, single nucleotide substitutions and insertions/deletions were determined by using GATK (Unified Genotyper) (DePristo et al., 2011).

Locations of all the variants were mapped using the National Center for Biotechnology Information (NCBI) and Ensembl databases. Amino acid change predictions and protein modeling were performed by using protein database resources as UniProt (UniProt Consortium, 2018) and Mutation Assessor (Mutation Assessor, Computational Biology Center, 2018).

2.3. Statistical analysis

For the bioinformatic analysis, sample results were analyzed and filtered. Significant variant filtering was performed according to the population frequency of the exome and whole-genome sequence data. All variants with a frequency of < 5% in the Exome Aggregation Consortium (ExAC) and 1000 Genome databases were filtered and compared in the study groups.

The results of the study were analyzed with the SPSS statistical program (IBM Corporation version 20.0 SPSS Inc., Chicago, IL, USA). Student's t-test and Mann–Whitney U test were applied to compare normally and non-normally distributed continuous variables between the study groups. Quantitative data were presented as means (± standart deviation (SD) or standart error (SEM)), while categorical data were presented as numbers and percentages. Gene counting method was used for the evaluation of allele frequency. Qualitative data such as genotypes and alleles comparison and consistency with Hardy-Weinberg Equilibrium (HWE) were tested using the Chi-square statistic. Odds ratio (OR) and 95% confidence interval (95% CI) were used to determine the risk factor between groups. A p value of < 0.05 was considered statistically significant.

Body surface area (BSA) was calculated according to the formula of Du Bois and Du Bois (Du Bois and Du Bois, 1989). BSA (m2) = 0,007184 × W0,425 × H0,725 where W: weight (kilogram), H: height (centimeter). Power analysis was performed using PS Power and Sample Size Calculation version 3.0 program (Dupont et al., 2018).

3. Results

3.1. Clinical Investigation

Demographic, biochemical and clinical data are summarized in Table 1. Body mass index (BMI) ($p < 0.001$), waist circumference ($p < 0.001$), blood glucose level ($p < 0.001$), hemoglobin A1c (HbA1c, %) ($p < 0.001$), triglyceride ($p < 0.001$), VLDL-cholesterol ($p = 0.018$), C-reactive protein (CRP) ($p < 0.001$), thyroid-stimulating hormone (TSH) ($p = 0.043$), free thyroxine (fT4) ($p = 0.006$), uric acid ($p = 0.03$), urea ($p = 0.002$), blood urea nitrogen (BUN) ($p < 0.001$), and presence of family history of diabetes ($p < 0.001$) were higher in T2DM group than those of the control group, while HDL-cholesterol level ($p < 0.001$) and CKD-EPI formula (Chronic Kidney Disease Epidemiology Cooperation) value ($p < 0.001$) were lower.

3.2. Sequence variants in Rev erb alpha and Rev erb beta genes

Rare genetic variations detected in *Rev-erb alpha* and *Rev-erb beta* genes in the control and the T2DM groups. The chromosomal locations, amino acid changes, protein regions, functional effects, SIFT and Polyphen2 scores, and minor allele frequencies of these genetic variations of were listed in (Suppl. Table 1). Among rare mutations; 13 missense mutations, 9 silent mutations, three 5′UTR variations, and one 3′UTR variation were detected. Of these 26 variations, 9 novel mutations (5 missense and 3 silent and 1 5′UTR) were detected (Suppl. Table 1).

Common variations in *Rev-erb alpha* and *Rev-erb beta* genes in our study groups were *Rev-erb alpha* Chr17:38253751 (T > C), *Rev-erb alpha* rs72836608 (C > A), *Rev-erb alpha* rs2314339 (C > T), *Rev-erb alpha* rs2102928 (C > T), *Rev-erb beta* Chr3:24003765 (A > G), and *Rev-erb beta* rs924403442 (Chr3:24006717 (G > T)). As shown in Table 2, no significant differences were detected in the genotypes and alleles distribution of these exonic and intronic variations of *Rev-erb alpha* and *Rev-erb beta* genes between T2DM patient and control groups ($p > 0.05$). No deviation from HWE ($p > 0.05$) was detected in both patient and control groups for the *Rev-erb beta* Chr3:24003765 (A > G), *Rev-erb alpha* rs72836608 (C > A), *Rev-erb alpha* rs2314339 (C > T) and *Rev-erb alfa* rs2102928 (C > T) variations except for *Rev-erb beta* rs924403442 (G > T) and *Rev-erb alpha* Chr17:38253751 (T > C) ($p < 0.05$). The distribution of these polymorphisms in the *Rev-erb alpha* and *Rev-erb beta* genes were similar between the control group and both obese and nonobese T2DM patient subgroups, female and male subgroups, and between the T2DM patient subgroups (obese vs. non-obese) ($p > 0.05$) except for *Rev-erb beta* Chr3:24003765 SNP. In the male controls, it was observed that *Rev-erb beta* Chr3:24003765 heterozygote AG genotype frequency was higher (44.8% vs. 10.8%; $p = 0.004$, Fisher exact test), and the normal AA genotype was lower than female controls (Table 2).

Table 1
Characteristics of the study population.

Parameters	CONTROL (n = 66)	T2DM (n = 42)	p value
Demographic			
Age (year)	59.0 ± 3.0	59.2 ± 1.3	0.188
Sex (Female/Male) (n)	37/29	25/17	0.723
Smoking (%)	29.3	17.5	0.211
SBP (mm Hg)	116.9 ± 2.0	133.8 ± 9.8	0.132
DBP (mm Hg)	75.8 ± 1.4	81.9 ± 4.2	0.125
Family history of diabetes (%)	25.6	76.5	**0.001**
Glucose (mg/dL)	89.2 ± 1.2	161.3 ± 9.1	**0.001**
HbA1c (%)	5.5 ± 0.1	7.6 ± 0.1	**0.001**
TC (mg/dL)	198.5 ± 5.9	202.5 ± 8.2	0.681
TG (mg/dL)	115.4 ± 8.7	183.6 ± 20.2	**0.001**
HDL-C (mg/dL)	51.7 ± 1.8	40.9 ± 2.0	**0.001**
LDL-C (mg/dL)	117.4 ± 4.5	127.2 ± 6.5	0.204
VLDL-C (mg/dL)	24.1 ± 1.9	33.8 ± 4.0	**0.018**
BMI (kg/m^2)	25.6 ± 0.5	30.5 ± 0.8	**0.001**
BSA (m2)	1.8 ± 0.1	1.8 ± 0.1	0.528
Waist circumference (cm)	82.2 ± 3.4	105.0 ± 2.1	**0.001**
Liver function			
ALT (U/L)	18.6 ± 1.5	21.7 ± 1.4	0.152
AST (U/L)	17.4 ± 0.6	23.9 ± 4.9	0.198
GGT (U/L)	16.5 ± 4.1	32.7 ± 11.6	0.331
CRP (mg/dL)	0.4 ± 0.2	3.3 ± 0.7	**0.001**
Kidney function			
BUN (mg/dL)	12.4 ± 0.5	16.1 ± 1.0	**0.001**
Uric acid (mg/dL)	4.8 ± 0.3	5.8 ± 0.3	**0.03**
Urea (mg/dL)	26.4 ± 1.2	37.1 ± 3.0	**0.002**
Creatinine (mg/dL)	0.8 ± 0.1	0.9 ± 0.07	0.090
Microalbuminuria (mg/L)	–	32.6 ± 9.5	
CKD-EPI	110.3 ± 4.8	87.7 ± 3.1	**0.001**

The results are shown as X (mean) ± SD (standard deviation). Significance bewtween groups was analyzed by Student's t test. n: number of individuals, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, HbA1c: Hemoglobin A1c, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol, BMI: Body mass index, BSA: Body Surface Area, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma glutamyl transferase, CRP: C-reactive protein, BUN: Blood urea nitrogen, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration formula value (mL/min/ 1.73 m^2).

3.3. Effects of the common variations of the Rev erb-alpha and Rev erb beta genes on metabolic parameters

Table 3 shows the association of Rev-erb alpha common genotypes and alleles with biochemical parameters in the study groups. Accordingly, in the total T2DM group, it was observed that serum HDL-C levels were lower in patients carrying *Rev-erb alpha* rs72836608 (C > A) A allele ($p = 0.025$) and *Rev-erb alpha* rs2314339 (C > T) CC genotype ($p = 0.027$) than in those with rs72836608 CC genotype and rs2314339 T allele, respectively. Patients with *Rev-erb alpha* rs2102928 (C > T) homozygous CC genotype had higher microalbuminuria level ($p = 0.035$) and SBP ($p = 0.043$) than those with rare T allele (TT + CT genotypes). In the control group, *Rev-erb alpha* Chr17:38253751 minor C allele carriers (CC + CT genotypes) had higher waist circumference (p = 0.014) and lower DBP (p = 0.024),

Table 2
The genotype and allele distributions of the common *Rev-erb alpha* and *Rev-erb beta* genetic variations in the study groups.

			CONTROL			T2DM				
			Total (n = 66)	Women (n = 37)	Men (n = 29)	Total (n = 42)	Women (n = 25)	Men (n = 17)	Obese T2DM (n = 21)	Non-obese T2DM (n = 21)
REV-ERB ALPHA CHR17:38253751 T > C INTRONIC	Genotypes, % (n)	TT	42.4 (28)	45.9 (17)	37.9 (11)	38.1 (16)	44.0 (11)	29.4 (5)	47.6 (10)	28.6 (6)
		TC	57.6 (38)	54.1 (20)	62.1 (18)	61.9 (26)	56.0 (14)	70.6 (12)	52.4 (11)	71.4 (15)
	Alleles, % (n)	T	71.2 (94)	73.0 (54)	69.0 (40)	69 (58)	72.0 (36)	64.7 (22)	73.8 (31)	64.3 (27)
		C	28.8 (38)	27.0 (20)	31.0 (18)	31 (26)	28.0 (14)	35.3(12)	26.2(11)	35.7 (15)
	HWE		$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$
REV-ERB ALPHA RS72836608 C > A INTRONIC	Genotypes, % (n)	CC	50 (33)	45.9 (/17)	55.2 (16)	45.2 (19)	44.0 (11)	47.1 (8)	52.4 (1 1)	38.1 (8)
		AA	9.1 (6)	8.1 (3)	10.3 (3)	9.5 (4)	4.0 (1)	17.6 (3)	9.5 (2)	9.5 (2)
		CA	40.9 (27)	45.9 (17)	34.5 (10)	45.2 (19)	52.0 (13)	35.3 (6)	38.1 (8)	52.4 (11)
	Alleles, % (n)	C	70.5 (93)	68.9 (51)	72.4(42)	67.9 (57)	70.0 (35)	64.7 (22)	71.4 (30)	64.3 (27)
		A	29.5 (39)	31.1 (23)	27.6(16)	32.1 (27)	30.0(15)	35.0 (12)	28.6 (12)	35.7 (15)
	HWE		$p > 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$
REV-ERB ALPHA RS2314339 C > T INTRONIC	Genotypes, % (n)	CC	66.6 (40)	56.8 (21)	65.5 (15)	66.7 (28)	56.0 (14)	82.4 (14)	61.9 (13)	71.4 (15)
		TT	3 (2)	2.7 (1)	3.4 (1)	4.8 (2)	8.0 (2)	0	0	9.5 (2)
		CT	36.4 (24)	40.5 (15)	31.0 (9)	28.6 (12)	36.0 (9)	17.6 (3)	38.1 (8)	19.0 (4)
	Alleles, % (n)	C	78.8 (1 0 4)	77.0 (57)	67.2 (39)	81 (68)	74.0 (37)	91.2 (31)	81.0 (34)	81.0 (34)
		T	21.2 (28)	23.0 (17)	(11)	19 (16)	26.0 (13)	8.8 (3)	19.0 (8)	19.0 (8)
	HWE		$p > 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$
REV-ERB ALPHA RS2102928 C > T INTRONIC	Genotypes, % (n)	CC	39.4 (26)	40.5 (15)	37.9 (11)	40.5 (17)	40.0 (10)	41.2 (7)	9.5 (2)	14.3 (3)
		TT	10.6 (7)	5.4 (2)	17.2 (5)	11.9 (5)	4.0 (1)	23.5 (4)	42.9 (9)	38.1 (8)
		CT	50 (33)	54.1 (20)	44.8 (13)	47.6 (20)	56.0 (14)	35.3 (6)	47.6 (10)	47.6 (10)
	Alleles, % (n)	C	64.4 (85)	67.6 (50)	60.3 (35)	64.3 (54)	68.0 (34)	58.8 (20)	33.3 (14)	38.1 (16)
		T	35.6 (47)	32.4 (24)	39.7 (23)	35.7 (30)	32.0 (16)	41.2 (14)	66.7 (28)	61.9 (26)
	HWE		$p > 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$
REV-ERB BETA CHR3:24003765 A > G (Q197R) EXON 5	Genotypes, % (n)	AA	74.2 (49)	89.2 (33)	55.2 (16)	71.4 (30)	72.0 (18)	70.6 (12)	66.7 (14)	76.2 (16)
		AG	25.8 (17)	10.8 (4)	**44.8 (13) a**	28.6 (12)	28.0 (7)	29.4 (5)	33.3 (7)	23.8 (5)
	Alleles, % (n)	A	87.1 (1 1 5)	94.6 (70)	77.6 (45)	85.7 (72)	86.0 (43)	85.3 (29)	83.3 (35)	88.1 (37)
		G	12.9 (17)	5.4 (4)	22.4 (13)	14.3 (12)	14.0 (7)	14.7 (5)	16.7 (7)	11.9 (5)
	HWE		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
REV-ERB BETA RS924403442 G > T INTRONIC	Genotypes, % (n)	GG	42.4 (28)	40.5 (15)	44.8 (13)	50 (21)	56.0 (14)	41.2 (7)	57.1 (12)	42.9 (9)
		GT	57.6 (38)	59.5 (22)	55.2 (16)	50 (21)	44.0 (11)	58.8 (10)	42.9 (9)	57.1 (12)
	Alleles, % (n)	G	71.2 (94)	70.3 (52)	72.4 (42)	75 (63)	78.0 (39)	70.6 (24)	78.6 (33)	71.4 (30)
		T	28.8 (38)	29.7 (22)	27.6 (16)	25 (21)	22.0 (11)	29.4 (10)	21.4 (9)	28.6 (12)
	HWE		$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

HWE: Hardy-Weinberg Equilibrium.
a, p = 0.004 (Fisher's exact test).

HbA1c (%) ($p = 0.002$), serum AST ($p = 0.045$) and LDL-C level ($p < 0.05$) than those with *Rev-erb alpha* homozygote TT genotype. The serum creatinine levels were significantly lower in control subjects with *Rev-erb alpha* rs72836608 A allele than those with the CC homozygote genotype ($p = 0.009$) (Table 3). Also, subjects carrying *Rev-erb alpha* rs2314339 T allele showed a lower free thyroxine hormone level than those with the CC homozygote genotype ($p = 0.037$) in the control group (data not shown).

The association of *Rev-erb beta* common genotypes and alleles with biochemical parameters in the study groups were presented in Table 4. The control subjects carrying *Rev-erb beta Chr3:*24003765 (A > G) G allele had higher BSA ($p = 0.03$), creatinine ($p < 0.001$), and ALT levels ($p = 0.009$) than those with AA genotype. Also, in both patient and control groups, *Rev-erb beta* Chr3:24003765 G allele carriers had higher serum TG (~35%) and lower HDL-C (~11%) than those with AA genotype close to statistically significant. T2DM patients with *Rev-erb beta* Chr3:24003765 G allele had higher TSH levels ($p = 0.028$) than those with AA genotype (data not shown). *Rev-erb beta* rs924403442 (G > T) T allele compared to the GG genotype was found to be associated with low BSA in the T2DM group ($p = 0.039$) and lower ALT levels in the control group ($p = 0.027$) (Table 4).

In our study, 21 of T2DM patients were *obese* (median BMI: 32.89, Q1-Q3: 32.06–36.35), and 21 were *non-obese* (median BMI: 26.80, Q1-Q3: 25.25–28.04). When the statistical analysis performed according to obese T2DM and non-obese T2DM subgroups, it was found that the diabetes duration ($p < 0.001$), and male gender ($p < 0.01$) were higher in obese T2DM compared to non-obese patients. The obese T2DM patient group consists of non-smokers ($p = 0.008$). As expected, waist circumference and BMI values were also higher in obese T2DMs than non-obese T2DMs ($p < 0.001$). Hovewer, there were no significant differences between the obese and non-obese T2DM subgroups in terms of other biochemical parameters ($p > 0.05$) (Table 5).

When we examined the effects of *Rev-erb* gene variations in the *obese* T2DM patient subgroup, microalbuminuria level was higher in patients carrying *Rev-erb alpha* rs72836608 (C > A) CC genotype compared to A allele. In patients with *Rev-erb alpha* rs2314339 (C > T) T allele, serum level of GGT was higher than those with CC genotype ($p = 0.048$). Moreover, *Rev-erb alpha* rs2102928 (C > T) homozygote

Table 3
The effects of *Rev-erb alpha* common genotypes and alleles on biochemical parameters in the study groups.

Parameters		*Rev-erb alpha* Chr17:38253751 T > C				*Rev-erb alpha* rs72836608 C > A		
		Control TT (n = 28)	TC+CC (n = 38)	Type 2 DM TT (n = 16)	TC+CC (n = 26)	Control CC (n = 33)	CA+AA (n = 33)	Type 2 DM CC (n = 19)
Demographic	SBP (mm Hg)	118.9 ± 4.0	115.6 ± 1.8	115.0 ± 5.0	140.0 ± 12.1	118.2 ± 2.9	115.4 ± 2.4	146.7 ± 18.6
	DBP (mm Hg)	**79.7 ± 2.2 b**	73.2 ± 1.8	80.0 ± 0.0	82.5 ± 5.7	76.5 ± 2.0	75.0 ± 2.1	80.0 ± 10.0
	Glucose (mg/dL)	91.0 ± 2.1	87.8 ± 1.5	161.4 ± 14.8	161.3 ± 11.7	89.6 ± 1.7	88.8 ± 1.8	152.4 ± 8.1
	HbA1c (%)	**5.7 ± 0.1 b**	5.4 ± 0.1	7.7 ± 0.2	7.6 ± 0.2	5.6 ± 0.1	5.5 ± 0.0	7.7 ± 0.2
	TC (mg/dL)	209.4 ± 10.5	190.4 ± 6.5	205.4 ± 13.4	200.8 ± 10.6	200.9 ± 8.0	195.9 ± 8.8	211.8 ± 10.6
	TG (mg/dL)	129.9 ± 15.6	103.4 ± 9.1	194.0 ± 41.5	177.2 ± 21.1	117.0 ± 13.3	113.8 ± 11.5	175.4 ± 15.1
	HDL-C (mg/dL)	52.6 ± 3.1	51.1 ± 2.1	41.6 ± 3.4	40.5 ± 2.4	53.0 ± 2.5	50.4 ± 2.5	45.6 ± 2.2
	LDL-C (mg/dL)	**127.6 ± 7.6 c**	109.3 ± 5.2	127.8 ± 10.1	126.8 ± 8.6	118.7 ± 5.7	116.1 ± 7.2	136.0 ± 9.9
	VLDL-C (mg/dL)	27.4 ± 3.2	21.6 ± 2.3	36.0 ± 8.3	32.4 ± 4.0	24.0 ± 2.6	24.1 ± 2.8	30.2 ± 2.1
	BMI (kg/m^2)	25.7 ± 1.0	25.5 ± 0.6	30.9 ± 1.2	30.3 ± 1.1	24.9 ± 0.7	26.3 ± 0.8	32.0 ± 1.4
	BSA (m^2)	1.8 ± 0.0	1.9 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0
	WC (cm)	74.8 ± 2.7	**90.7 ± 5.1 d**	105.9 ± 2.3	104.4 ± 3.2	81.3 ± 5.0	83.0 ± 5.1	107.1 ± 3.1
Liver Function	ALT (U/L)	21.0 ± 2.5	16.6 ± 1.7	20.8 ± 1.8	22.3 ± 2.1	19.7 ± 2.1	17.2 ± 2.1	19.9 ± 2.2
	AST (U/L)	**18.7 ± 0.9 e**	16.3 ± 0.8	17.3 ± 1.2	27.7 ± 7.6	17.8 ± 0.9	17.0 ± 0.9	29.3 ± 10.7
	GGT (U/L)	10.8 ± 0.5	22.3 ± 7.3	21.8 ± 4.4	40.0 ± 19.2	23.8 ± 6.3	9.3 ± 1.1	48.4 ± 24.1
	CRP (mg/dL)	0.6 ± 0.3	0.2 ± 0.1	3.6 ± 1.6	3.2 ± 0.8	0.3 ± 0.1	0.6 ± 0.3	3.7 ± 1.7
Kidney Function	BUN (mg/dL)	12.7 ± 0.9	12.1 ± 0.7	16.2 ± 1.1	15.9 ± 1.5	13.0 ± 0.8	11.7 ± 0.7	15.6 ± 1.4
	Uric acid (mg/dL)	4.49 ± 0.29	5.2 ± 0.4	6.1 ± 0.3	5.6 ± 0.4	4.9 ± 0.7	4.8 ± 0.3	6.2 ± 0.4
	Urea (mg/dL)	27.3 ± 1.8	25.5 ± 1.5	34.3 ± 2.3	38.8 ± 4.7	27.9 ± 1.8	24.9 ± 1.5	39.7 ± 5.7
	Creatinine (mg/dL)	0.8 ± 0.0	0.80 ± 0.03	0.8 ± 0.1	1.0 ± 0.1	**0.8 ± 0.0 f**	0.7 ± 0.0	1.1 ± 0.2
	Malb (mg/L)			17.1 ± 6.8	42.4 ± 14.7	-	-	49.3 ± 18.5
	CKD-EPI	109.2 ± 5.9	112.4 ± 9.2	85.6 ± 6.3	88.8 ± 3.4	107.9 ± 5.0	112.3 ± 8.1	83.7 ± 4.8

Parameters	*Rev-erb alpha* rs72836608 C > A	*Rev-erb alpha* rs2314339 C > T				*Rev-erb alpha* rs2102928 C > T			
	Type 2 DM CA+AA (n = 23)	Control CC (n = 40)	CT+TT (n = 26)	Type 2 DM CC (n = 28)	CT+TT (n = 14)	Control CC (n = 26)	CT+TT (n = 40)	Type 2 DM CC (n = 17)	CT+TT (n = 25)
Demographic	126.0 ± 11.2	117.7 ± 3.0	115.9 ± 2.1	131.7 ± 12.2	140.0 ± 20.0	117.0 ± 3.6	116.8 ± 2.1	**152.5 ± 14.4 i**	115.0 ± 2.9
	83.0 ± 4.4	77.0 ± 1.9	74.2 ± 2.2	80.8 ± 5.5	85.0 ± 5.0	75.1 ± 2.5	76.2 ± 1.8	85.0 ± 8.7	78.8 ± 1.3
	168.7 ± 15.2	88.5 ± 1.4	90.3 ± 2.3	157.6 ± 8.8	168.8 ± 21.2	87.6 ± 1.8	90.2 ± 1.7	143.4 ± 7.9	173.6 ± 13.9
	7.6+ ± 0.2	5.5 ± 0.0	5.5 ± 0.1	7.5 ± 0.2	7.8 ± 0.2	5.5 ± 0.1	5.5 ± 0.0	7.5 ± 0.2	7.7 ± 0.2
	194.9 ± 12.2	199.1 ± 7.9	197.5 ± 8.8	200.7 ± 11.0	206.2 ± 11.7	197.6 ± 9.0	199.0 ± 7.8	200.0 ± 10.9	204.3 ± 11.8
	190.4 ± 35.1	116.2 ± 12.2	114.0 ± 11.8	193.9 ± 29.6	163.0 ± 14.0	115.5 ± 15.7	115.3 ± 10.4	164.9 ± 13.1	196.4 ± 32.9
	37.0 ± 2.9 g	51.2 ± 2.6	52.5 ± 2.3	**37.9 ± 2.6 h**	46.9 ± 1.7	53.7 ± 3.2	50.4 ± 2.1	44.6 ± 2.7	38.4 ± 2.6
	120.0 ± 8.5	119.2 ± 6.3	114.7 ± 6.3	125.8 ± 7.9	130.0 ± 11.7	115.1 ± 6.2	118.9 ± 6.4	125.4 ± 9.8	128.4 ± 8.7
	36.7 ± 7.1	24.6 ± 2.7	23.2 ± 2.4	36.3 ± 5.8	28.6 ± 2.0	23.6 ± 3.1	24.4 ± 2.4	30.0 ± 2.0	36.3 ± 6.5
	29.3 ± 0.9	25.7 ± 0.7	25.4 ± 0.8	29.6 ± 0.8	32.4 ± 1.8	24.8 ± 0.8	26.1 ± 0.7	30.9 ± 1.3	30.3 ± 1.1
	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.1	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.9 ± 0.0
	103.1 ± 2.9	79.4 ± 3.2	87.8 ± 8.1	102.1 ± 2.2	110.2 ± 4.1	80.3 ± 5.7	83.4 ± 4.5	104.8 ± 3.9	105.1 ± 2.5
Liver Function	23.0 ± 1.9	17.9 ± 2.0	19.7 ± 2.3	21.8 ± 1.8	21.5 ± 2.5	18.1 ± 2.0	19.0 ± 2.1	20.6 ± 2.2	22.3 ± 1.9
	19.3 ± 1.1	17.4 ± 0.7	17.5 ± 1.1	26.4 ± 7.6	19.5 ± 2.8	16.8 ± 0.9	17.8 ± 0.8	28.9 ± 11.4	20.2 ± 1.8
	19.0 ± 3.3	18.0 ± 4.3	6.0 ± 0.0	19.1 ± 2.7	60.0 ± 33.2	23.8 ± 6.3	9.3 ± 1.1	22.1 ± 3.8	42.0 ± 21.6
	3.1 ± 0.7	0.5 ± 0.2	0.2 ± 0.1	3.1 ± 0.6	3.8 ± 2.1	0.3 ± 0.1	0.5 ± 0.3	2.1 ± 0.7	3.6 ± 0.9

(*continued on next page*)

Table 3 (*continued*)

Parameters	*Rev-erb alpha* rs72836608 C > A	*Rev-erb alpha* rs2314339 C > T			*Rev-erb alpha* rs2102928 C > T			
	Type 2 DM CA+AA (n = 23)	Control CC (n = 40)	CT+TT (n = 26)	Type 2 DM CC (n = 28)	CT+TT (n = 14)	Control CC (n = 26)	CT+TT (n = 40)	Type 2 DM CC (n = 17)	CT+TT (n = 25)
Kidney Function	16.3 ± 1.4	12.7 ± 0.7	11.9 ± 0.9	16.0 ± 1.0	16.0 ± 2.5	12.3 ± 1.0	12.4 ± 0.6	15.5 ± 1.4	16.3 ± 1.4
	5.4 ± 0.4	4.6 ± 0.3	5.2 ± 0.4	5.9 ± 0.4	5.6 ± 0.5	5.1 ± 0.5	4.7 ± 0.3	6.4 ± 0.5	5.4 ± 0.4
	34.9 ± 2.8	26.9 ± 1.5	25.6 ± 1.9	37.4 ± 4.0	36.5 ± 4.4	26.6 ± 2.1	26.3 ± 1.4	40.1 ± 6.7	35.3 ± 2.7
	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	1.0 ± 0.2	0.8 ± 0.1
	17.0 ± 4.2			25.0 ± 9.0	46.6 ± 21.4	-	-	**57.5 ± 19.1 j**	12.2 ± 3.0
	91.0 ± 3.9	107.9 ± 4.6	134.5 ± 0.1	86.2 ± 3.5	90.6 ± 6.1	109.6 ± 6.0	110.8 ± 7.0	86.85.0	88.2 ± 4.0

The results are shown as mean ± SD (standard deviation). SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, HbA1c: Hemoglobin A1c, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma glutamyl transferase, BMI: Body mass index, BSA: Body Surface Area, WC: Waist circumference. CRP: C-reactive protein, BUN: Blood urea nitrogen, Malb: Microalbuminuria CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration formula value (mL/min/1.73 m^2).
Bold values indicate statistical significance. a, $p = 0.024$; b, $p = 0.002$; c, $p < 0.05$; d, $p = 0.014$; e, $p = 0.045$; f, $p = 0.009$; g, $p = 0.025$; h, $p = 0.027$; i, $p = 0.043$; j, $p = 0.035$.

common CC genotype was observed to be associated with the increased uric acid ($p < 0.05$) and microalbuminuria ($p = 0.006$) and rare T allele was asociated decreased HDL-C ($p = 0.023$) levels (Table 6). On the other hand, no relationship was observed between *Rev-erb alpha* Chr17:38253751 (T > C) and biochemical parameters in *non-obese* T2DM patients ($p > 0.05$). Similar to obese T2DMs, *Rev-erb alpha* rs72836608 (C > A) CC genotype has higher levels of microalbuminuria than A allele in *non-obese* T2DM subgroup (52.80 ± 43.50 vs. 24.91 ± 6.77), but the relationship was not statistically significant. *Rev-erb alpha* rs2314339 homozygous CC genotype was associated with decreased HDL-C compared to the T allele ($p = 0.036$). *Rev-erb alpha* rs2102928 T allele was found to be associated with increased fasting blood glucose levels ($p < 0.05$) (Table 7).

When we examined the effect of *Rev-erb beta* gene variations on biochemical parameters in obese and non-obese T2DMs, we found an important effect of rare G allele of *Rev-erb beta* Chr3:24003765 A > G mutation on increased serum GGT levels in obese T2DM group. However, this association was not observed non-obese T2DM group ($p > 0.05$). In the non-obese T2DM subgroup, the GG genotype of *Rev-erb beta* rs924403442 G > T is associated with a high BSA value compared to the T allele ($p = 0.049$, Table 8), as in T2DM total patient group (*obese* + *non-obese*) (Table 3). In contrast, this relationship was not observed in the obese T2DM subgroup ($p > 0.05$).

When study parameters are examined according to gender, waist circumference ($p = 0.002$), ALT ($p < 0.001$), AST ($p < 0.001$), uric acid ($p = 0.012$), urea ($p < 0.001$), creatinine ($p < 0.001$), BUN ($p = 0.002$) and BSA values ($p = 0.01$) in male controls were higher than females, while HDL-C level was lower ($p < 0.001$).

In the total T2DM patient group, male patients had higher smoking ($p = 0.013$), alcohol consumption ($p = 0.005$), urea ($p = 0.039$), creatinine ($p = 0.006$), BUN ($p = 0.008$) and BSA values ($p = 0.017$) than women, while BMI ($p = 0.001$), waist circumference ($p = 0.044$) and HDL-C levels ($p = 0.011$) were lower than female patients (Suppl. Table 2).

We also examined the effects of common *Rev-erb alpha* SNPs on biochemical parameters in gender subgroups in the total T2DM group. In male T2DM patients carrying rare A allele of rs72836608 (C > A) ($p = 0.036$) and homozygous CC genotype of rs2314339 (C > T) ($p = 0.047$), serum HDL-C values were lower than those carrying the rs72836608 CC genotpe and rs2314339 T allele, respectively. In male patients carrying T allele of *Rev-erb alpha* rs2102928 (C > T), fasting blood glucose level was observed higher ($p = 0.019$) (Suppl. Table 3). While the effects of *Rev-erb alpha* SNPs on HDL-C and fasting blood glucose levels were not observed in female patients, increased microalbuminuria levels were found in female patients with homozygous CC genotype of *Rev-erb alpha* rs2102928 (C > T) SNP ($p = 0.008$) (Suppl. Table 4).

Rev-erb beta SNPs also had different effects by gender in the T2DM patient group. In female patients, *Rev-erb beta* rs924403442 (G > T) homozygous GG genotype is associated with low HDL-C ($p = 0.013$) and high BSA ($p = 0.044$) values compared to the rs924403442 T allele. On the other hand, Chr3:24003765 (A > G), localized in exon 5, did not have a significant effect in both female and male subgroups ($p > 0.05$) (Suppl. Table 5). *Rev-erb beta* Chr3:24003765 heterozygous AG genotype, which was observed in male control subgroup more frequently than common AA genotype ($p = 0.004$), was not associated with studied biochemical and other parameters ($p > 0.05$). On the other hand, the relation of the Chr3:24003765 G allele with the creatinine levels in the total control group was also observed in female controls (for creatinine levels, G allele: 0.89 ± 0.08 vs. AA genotype: 0.68 ± 0.02, $p = 0.005$) (data not shown).

3.4. Power analysis

The statistical power analysis of the association between *Rev-erb alpha* Chr17:38253751 T > C variation localized in intron 4 and the

Table 4
The effects of *Rev-erb beta* frequent genotypes and alleles on biochemical parameters in the study groups.

Parameters		*Rev-erb beta* Chr3:24003765 A > G (Q197R) exon 5				*Rev-erb beta* rs924403442 (Chr3:24006717) G > T			
		Control		Type 2 DM		Control		Type 2 DM	
		AA (n = 49)	AG + GG (n = 17)	AA (n = 30)	AG + GG (n = 12)	GG (n = 28)	GT + TT (n = 38)	GG (n = 21)	GT + TT (n = 21)
Demographic	SBP (mm Hg)	116.7 ± 2.5	117.3 ± 2.2	133.3 ± 11.7	135.0 ± 25.0	118.6 ± 3.4	115.5 ± 2.1	150.0 ± 15.3	124.0 ± 11.7
	DBP (mm Hg)	75.7 ± 1.8	76.1 ± 2.2	84.2 ± 3.8	75.0 ± 15.0	76.7 ± 2.5	75.1 ± 1.7	86.7 ± 3.3	79.0 ± 6.4
	Glucose (mg/dL)	88.6 ± 1.5	90.9 ± 2.2	162.9 ± 11.6	157.5 ± 13.3	90.1 ± 2.0	88.6 ± 1.6	150.9 ± 10.9	171.8 ± 14.4
	HbA1c (%)	5.5 ± 0.0	5.6 ± 0.1	7.6 ± 0.2	7.8 ± 0.3	5.6 ± 0.1	5.5 ± 0.0	7.6 ± 0.2	7.6 ± 0.2
	TC(mg/dL)	200.1 ± 6.9	194.3 ± 11.5	200.4 ± 9.6	207.8 ± 16.4	203.8 ± 8.9	194.3 ± 7.9	201.1 ± 11.2	204.0 ± 12.3
	TG (mg/dL)	105.1 ± 8.9	142.5 ± 20.3	171.7 ± 23.2	213.3 ± 40.9	127.9 ± 14.6	105.8 ± 10.4	195.9 ± 32.3	171.3 ± 24.9
	HDL-C (mg/dL)	53.3 ± 2.2	47.3 ± 2.2	42.4 ± 2.3	37.1 ± 3.6	52.0 ± 2.9	51.5 ± 2.3	39.7 ± 2.3	42.1 ± 3.2
	LDL-C (mg/dL)	117.0 ± 5.5	118.5 ± 8.0	125.3 ± 7.5	132.1 ± 13.0	121.5 ± 6.1	114.1 ± 6.6	125.7 ± 9.2	128.7 ± 9.4
	VLDL-C (mg/dL)	23.1 ± 2.1	26.7 ± 4.2	32.1 ± 4.6	38.0 ± 8.0	26.8 ± 3.3	21.9 ± 2.1	35.3 ± 6.2	32.2 ± 5.0
	TC/HDL	4.1 ± 0.2	4.1 ± 0.2	7.0 ± 2.4	10.0 ± 4.9	4.1 ± 0.3	4.1 ± 0.3	8.2 ± 3.4	7.5 ± 2.8
	BMI (kg/m^2)	25.2 ± 0.7	26.5 ± 0.8	30.2 ± 1.0	31.4 ± 1.6	26.4 ± 0.8	24.9 ± 0.7	31.4 ± 1.2	29.6 ± 1.1
	BSA (m^2)	1.8 ± 0.0	**1.9 ± 0.1 a**	1.8 ± 0.0	1.9 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	**1.9 ± 0.0 e**	1.8 ± 0.0
	WC (cm)	81.9 ± 3.7	87.0 ± 1.0	104.1 ± 2.8	107.1 ± 2.5	82.6 ± 4.7	82.0 ± 4.8	105.4 ± 2.8	104.5 ± 3.3
Liver Function	ALT (U/L)	15.5 ± 1.2	**26.9 ± 3.6b**	21.5 ± 1.7	22.1 ± 2.6	**23.0 ± 2.7 d**	15.8 ± 1.5	22.9 ± 2.4	20.5 ± 1.6
	AST (U/L)	16.7 ± 0.7	19.2 ± 1.3	24.4 ± 6.1	22.0 ± 4.2	18.2 ± 1.1	16.8 ± 0.7	29.7 ± 9.4	17.8 ± 1.0
	GGT (U/L)	13.1 ± 2.6	40.0 ± 0.0	19.2 ± 2.7	70.0 ± 40.7	18.7 ± 10.7	15.2 ± 3.1	46.6 ± 24.5	20.6 ± 3.1
	CRP (mg/dL)	0.5 ± 0.2	0.2 ± 0.1	3.1 ± 0.6	3.8 ± 2.2	0.3 ± 0.1	0.6 ± 0.3	3.4 ± 1.2	3.2 ± 0.9
Kidney Function	BUN (mg/dL)	12.0 ± 0.7	13.2 ± 0.5	16.1 ± 1.1	15.8 ± 2.4	12.3 ± 0.9	12.4 ± 0.7	16.0 ± 1.2	16.0 ± 1.6
	Uric acid (mg/dL)	4.8 ± 0.3	5.1 ± 0.8	5.7 ± 0.3	6.0 ± 1.0	4.9 ± 0.3	4.8 ± 0.4	5.4 ± 0.4	6.1 ± 0.4
	Urea (mg/dL)	25.8 ± 1.5	28.3 ± 1.1	38.1 ± 4.0	34.7 ± 4.0	26.4 ± 1.7	26.4 ± 1.6	39.9 ± 4.8	33.8 ± 3.3
	Creatinine (mg/dL)	0.7 ± 0.0	**0.9 ± 0.0c**	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	1.0 ± 0.1	0.9 ± 0.1
	Malb (mg/L)	–	–	30.2 ± 11.2	38.5 ± 19.1	–	–	22.9 ± 5.5	44.5 ± 20.0
	CKD-EPI	111.3 ± 5.2	101.3 ± 0.1	91.1 ± 2.4	78.9 ± 8.7	117.1 ± 7.8	106.5 ± 6.0	87.1 ± 4.8	88.4 ± 3.6

The results are shown as mean ± SD (standard deviation). SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, HbA1c: Hemoglobin A1c, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol, BMI: Body mass index, BSA: Body Surface Area, WC: Waist circumference, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma glutamyl transferase, CRP: C-reactive protein, BUN: Blood urea nitrogen, Malb: Microalbuminuria, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration formula value (mL/min/1.73 m^2).
Bold values indicate statistical significance. a, $p = 0.03$; b, $p = 0.009$; c, $p = 0.001$; d, $p = 0.027$; e, $p = 0.039$.

Table 5
Comparison of biochemical parameters in the T2DM patients presence on obesity.

Parameters		Non obese (n = 21)	Obese (n = 21)	*p* value
Demographic	Age (year)	60.6 ± 1.4	59.7 ± 1.2	0.579
	Sex (Female/Male, n)	17/4	8/13	**0.011**
	Smoking (%)	35	0	**0.008**
	Alcohol consumption (%)	15.8	10	0.589
	Family history of diabetes (%)	69.2	81	0.434
	Diabetes duration (year)	9.9 ± 1.9	19.7 ± 1.9	**0.001**
	SBP (mm Hg)	133.8 ± 9.8	155.0 ± 5.0	0.143
	DBP (mm Hg)	81.9 ± 4.2	88.0 ± 1.3	0.286
	Glucose (mg/dL)	174.8 ± 16.4	147.9 ± 7.1	0.521
	HbA1c (%)	7.3 ± 0.3	7.9 ± 0.2	0.114
	TC(mg/dL)	211.8 ± 12.9	193.3 ± 10.1	0.339
	TG (mg/dL)	206.5 ± 38.7	160.8 ± 11.4	0.970
	HDL-C (mg/dL)	39.3 ± 3.2	42.5 ± 2.3	0.850
	LDL-C (mg/dL)	132.2 ± 9.6	122.2 ± 8.8	0.538
	VLDL-C (mg/dL)	39.4 ± 7.6	28.1 ± 1.7	0.589
	BMI (kg/m^2)	26.4 ± 0.5	34.7 ± 0.9	**0.001**
	BSA (m^2)	1.8 ± 0.0	1.9 ± 0.0	0.105
	WC (cm)	97.0 ± 3.4	109.9 ± 2.1	**0.001**
Liver Function	ALT (U/L)	20.8 ± 2.6	22.3 ± 1.7	0.413
	AST (U/L)	29.1 ± 11.4	20.0 ± 1.8	0.872
	GGT (U/L)	18.8 ± 3.4	42.0 ± 19.0	0.388
	CRP (mg/dL)	2.0 ± 0.4	4.3 ± 1.1	0.059
Kidney Function	BUN (mg/dL)	17.2 ± 1.8	15.0 ± 1.0	0.411
	Uric acid (mg/dL)	5.6 ± 0.4	5.9 ± 0.5	0.536
	Urea (mg/dL)	42.6 ± 6.4	32.9 ± 1.8	0.370
	Creatinine (mg/dL)	1.0 ± 0.1	0.8 ± 0.1	0.664
	Malb (mg/L)	34.2 ± 14.4	31.6 ± 12.9	0.646
	CKD-EPI	94.0 ± 3.9	83.8 ± 4.1	0.112

The results are shown as mean ± SEM (standard error). SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, HbA1c: Hemoglobin A1c, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol, BMI: Body mass index, BSA: Body Surface Area, WC:Waist circumference, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma glutamyl transferase, CRP: C-reactive protein, BUN: Blood urea nitrogen, Malb: Microalbuminuria, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration formula value (mL/min/1.73 m^2). Bold values indicate statistical significance.

risk of nephropathy in T2DM patients was calculated as 75% with 95% confidence interval ($\alpha = 0.05$). The *Rev-erb alpha* Chr17:38253751C allele had an increased risk of nephropathy when compared to the common TT genotype carriers in the T2DM patients (OR = 2.667, 95%CI:0.892–7.976, $p = 0.056$) (data not shown). This finding supports the sufficiency of our sample size.

4. Discussion

Although the effects of Rev-erb alpha and Rev-erb beta nuclear receptors on carbohydrate and lipid metabolism are known, the effects of changes in genes encoding these proteins in related metabolic disorders have not been investigated sufficiently. Therefore, we aimed to determine the variations of *Rev-erb alpha* and *Rev-erb beta* genes with the next-generation sequencing and compare the possible variations with biochemical effects between T2DM patients with or without obesity and healthy controls.

Rev-erb alpha and Rev-erb beta nuclear receptors have been reported to repress transcription of genes involved in glucose and lipid metabolism (Ramakrishnan et al., 2009; Noshiro et al., 2007; Wang et al., 2008). Both Rev-erb alpha and Rev-erb beta which were closely related to bind to nuclear receptors such as nuclear receptor corepressor 1 and act as transcriptional repressors (Yin and Lazar, 2005). They are involved in many pathologies such as T2DM, inflammation, heart disease and cancer (Vaissiere et al., 2015).

As well-known that there is a strong association between T2DM and obesity (Freemantle et al., 2008). Diabetic dyslipidemia is characterized by high triglyceride levels and low HDL cholesterol levels (Schofield et al., 2016). Obesity is known to be associated with increased insulin resistance, high serum triglyceride and LDL cholesterol levels, and low HDL cholesterol levels (Schofield et al., 2016; Durrington, 2007). It has been reported that HDL lipoprotein protects pancreatic beta cells against apoptosis (Von Eckardstein and Widmann, 2014). On the other hand, insulin mediates the transfer of triglycerides to apoB in the liver and is also involved in the hydrolysis of triglycerides from VLDL by regulating lipoprotein lipase activity in addition to its inhibitory effect on lipolysis. An increase in the levels of free fatty acids in the circulation inhibits the activity of lipoprotein lipase and leads to an increase in triglyceride-rich lipoprotein levels. Hypertriglyceridemia observed in insulin resistance may result from high serum free fatty acid levels and decreased apoB degradation. These conditions increase serum VLDL-cholesterol levels as a result of both increased VLDL production and decreased liver uptake and clearance (Schofield et al., 2016). In the present study, the high triglyceride, total cholesterol, LDL-cholesterol, VLDL-cholesterol and low HDL-cholesterol levels that we observed in T2DM patients were compatible with the dyslipidemic profile and were similar in the subgroups of obese and non-obese T2DM patients.

The findings of the limited number of studies in the literature suggest that Rev-erb alpha and Rev-erb beta deficiency are associated with disruption of circadian rhythm and lipid metabolism in mice (Cho et al., 2012; Bugge et al., 2012). Ma et al. showed that hematopoietic deficiency of Rev-erb alpha accelerates atherogenesis in LDL receptor-deficient mice (Ma et al., 2013). Another study has shown that Rev-erb binds to many of the genes involved in cholesterol biosynthesis and directly represses their expression. In the study of Sitaula et al., it was reported that the expression of genes in the cholesterol biosynthesis pathway and plasma cholesterol levels decreased as a result of treatment with synthetic Rev-erb agonists in mice. In their studies, Rev-erb has been shown to act directly by binding to the genes involved in cholesterol biosynthesis and suppress their expression or indirectly suppress cholesterol synthesis by inhibiting the expression of sterol regulatory element binding transcription factor 2 (Srebf2) (Sitaula et al., 2015). On the other hand, it was known that Rev-erb alpha represses plasminogen activator inhibitor type 1 (PAI-1) which leads to atherogenesis (Wang et al., 2006). Cesari et al (Cesari et al., 2010) documented increased expression of PAI-1 in atherosclerosis.

Vieira et al. (Vieira et al., 2014b) reported that *Rev-erb alpha* gene expression in visceral adipose tissue of the obese population showed a positive correlation with waist circumference and BMI. Ruano et al. (Ruano et al., 2014) showed that *Rev-erb alpha* rs939347 promoter variation in Spanish obese men, including type 2 diabetics, was associated with higher BMI and waist circumference ($p < 0.05$), reporting the association of Rev-erb alpha with adipogenesis and they concluded that this variation can alter the expression or regulation of Rev-erb alpha and its target genes. Goumidi et al. showed that the T minor allele of the *Rev-erb alpha* rs2071427 SNP (located in intron 1) was associated with high BMI in three population-based studies including 3480 adolescents and adults ($p < 0.05$) (Goumidi et al., 2013). Nascimento et al. also reported that *Rev-erb alpha* rs2071427 and rs2071570 SNPs are associated with BMI and sleep duration in boys (Nascimento et al., 2018).

In Garaulet et al. study investigating the association between *Rev-erb alpha* gene variations and metabolic parameters, it was reported that

Table 6
The effects of *Rev-erb alpha* common genotypes and alleles on biochemical parameters in the T2DM patients with obesity.

Parameters		*Rev-erb alpha* Chr17:38253751 T > C		*Rev-erb alpha* rs72836608 C > A		*Rev-erb alpha* rs2314339 C > T		*Rev-erb alpha* rs2102928 C > T	
		TT (n = 10)	TC + CC (n = 11)	CC (n = 11)	CA + AA (n = 10)	CC (n = 13)	CT + TT (n = 8)	CC (n = 2)	CT + TT (n = 19)
Demographic	SBP (mm Hg)	115.0 ± 5.0	140.0 ± 12.1	146.7 ± 18.6	126.0 ± 11.2	131.7 ± 12.2	140.0 ± 20.0	152.5 ± 14.4	115.0 ± 2.9
	DBP (mm Hg)	80.0 ± 2.0	82.5 ± 5.7	80.0 ± 10.0	83.0 ± 4.4	80.8 ± 5.5	85.0 ± 5.0	85.0 ± 8.7	78.8 ± 1.3
	Glucose (mg/dL)	145.5 ± 13.6	150.1 ± 6.4	150.7 ± 11.1	144.8 ± 9.1	149.5 ± 8.3	145.4 ± 13.7	146.1 ± 11.6	149.3 ± 9.3
	HbA1c (%)	7.8 ± 0.3	7.9 ± 0.2	8.0 ± 0.2	7.8 ± 0.3	7.8 ± 0.2	8.1 ± 0.2	7.9 ± 0.2	7.9 ± 0.2
	TC (mg/dL)	193.6 ± 15.2	193.0 ± 14.3	212.0 ± 14.6	172.7 ± 11.4	189.2 ± 13.2	200.1 ± 16.5	215.8 ± 16.9	176.4 ± 10.5
	TG (mg/dL)	161.2 ± 18.7	160.4 ± 14.5	168.7 ± 17.9	152.0 ± 14.1	163.1 ± 16.8	157.0 ± 13.8	153.9 ± 15.0	165.9 ± 16.9
	HDL-C (mg/dL)	41.6 ± 3.3	43.3 ± 3.3	46.3 ± 3.3	38.2 ± 2.8	40.5 ± 3.6	45.6 ± 1.3	48.1 ± 3.8	**38.3 ± 2.3c**
	LDL-C (mg/dL)	123.4 ± 13.7	121.2 ± 12.0	137.5 ± 13.3	105.5 ± 9.3	120.3 ± 10.3	125.4 ± 16.9	140.6 ± 15.9	108.5 ± 8.4
	VLDL-C (mg/dL)	28.5 ± 2.6	27.8 ± 2.4	28.3 ± 2.4	28.0 ± 2.6	28.3 ± 2.3	28.0 ± 2.9	27.2 ± 2.5	28.8 ± 2.5
	BMI (kg/m^2)	34.0 ± 0.7	35.3 ± 1.5	35.8 ± 1.5	33.5 ± 0.6	33.5 ± 0.6	36.6 ± 1.9	34.6 ± 1.5	34.7 ± 1.1
	BSA (m^2)	1.8 ± 0.1	1.9 ± 0.0	1.9 ± 0.0	1.9 ± 0.0	1.9 ± 0.0	1.9 ± 0.1	1.8 ± 0.0	1.9 ± 0.0
	WC (cm)	107.9 ± 2.9	111.7 ± 3.0	112.2 ± 3.2	107.4 ± 2.4	106.9 ± 2.0	114.9 ± 3.9	111.3 ± 3.6	108.8 ± 2.5
Liver Function	ALT (U/L)	20.8 ± 2.5	23.6 ± 2.3	21.1 ± ± 2.6	23.5 ± 2.2	22.4 ± 2.0	22.1 ± 3.3	19.9 ± 2.9	23.9 ± 2.0
	AST (U/L)	17.3 ± 1.7	22.0 ± 2.7	20.6 ± 3.2	19.3 ± 1.4	18.6 ± 1.3	22.0 ± 3.9	17.9 ± 1.9	21.9 ± 2.8
	GGT (U/L)	22.2 ± 5.3	66.8 ± 41.8	53.2 ± 28.0	19.7 ± 6.4	19.7 ± 3.2	**86.7 ± 52.8b**	25.4 ± 4.3	62.8 ± 43.3
	CRP (mg/dL)	3.6 ± 1.6	5.1 ± 1.6	4.6 ± 1.9	4.1 ± 1.4	4.1 ± 1.1	4.8 ± 2.7	2.2 ± 1.2	5.1 ± 1.3
Kidney Function	BUN (mg/dL)	16.5 ± 1.7	13.6 ± 1.1	14.4 ± 1.5	15.5 ± 1.4	15.4 ± 1.2	14.1 ± 1.8	14.8 ± 1.7	15.1 ± 1.3
	Uric acid (mg/dL)	6.4 ± 0.5	5.7 ± 0.6	6.6 ± 0.5	5.0 ± 0.7	5.7 ± 0.6	6.0 ± 0.7	**6.8 ± 0.5 d**	5.1 ± 0.5
	Urea (mg/dL)	33.8 ± 3.0	33.0 ± 2.3	31.6 ± 2.7	34.2 ± 2.5	33.0 ± 2.2	32.6 ± 3.4	33.0 ± 3.2	32.8 ± 2.3
	Creatinine (mg/dL)	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
	Malb (mg/L)	18.9 ± 9.0	43.0 ± 23.2	**48.1 ± 21.1 a**	9.0 ± 3.7	12.0 ± 2.9	58.6 ± 28.7	**56.7 ± 25.1 e**	9.0 ± 2.9
	CKD-EPI	81.7 ± 7.3	85.6 ± 4.9	80.6 ± 6.3	87.1 ± 5.6	82.8 ± 4.7	85.4 ± 8.0	83.0 ± 6.4	84.5 ± 5.6

The results are shown as mean ± SEM (standard error). SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, HbA1c: Hemoglobin A1c, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol, BMI: Body mass index, BSA: Body Surface Area, WC: Waist circumference, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma glutamyl transferase, CRP: C-reactive protein, BUN: Blood urea nitrogen, Malb:Microalbuminuria,CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration formula value (mL/min/1.73 m^2).
Bold values indicate statistical significance. a, $p = 0.016$; b, $p = 0.048$; c, $p = 0.023$; d, $p = 0.05$; e, $p = 0.006$.

Table 7
The effects of *Rev-erb alpha* common genotypes and alleles on biochemical parameters in the T2DM patients without obesity.

Parameters		*Rev-erb alpha* 38253751 T > C		*Rev-erb alpha* rs72836608 C > A		*Rev-erb alpha* rs2314339 C > T		*Rev-erb alpha* rs2102928 C > T	
		TT (n = 6)	TC + CC (n = 15)	CC (n = 8)	CA + AA (n = 13)	CC (n = 15)	CT + TT (n = 6)	CC (n = 3)	CT + TT (n = 18)
Demographic	SBP (mm Hg)	115.0 ± 5.0	140.0 ± 12.1	146.7 ± 18.6	126.0 ± 11.2	131.7 ± 12.2	140.0 ± 20.0	152.5 ± 14.4	115.0 ± 2.9
	DBP (mm Hg)	80.0 ± 0.2	82.5 ± 5.7	80.0 ± 10.0	82.0 ± 4.4	80.8 ± 5.5	85.0 ± 5.0	85.0 ± 8.7	78.8 ± 1.3
	Glucose (mg/dL)	188.0 ± 31.1	169.5 ± 19.8	154.6 ± 12.4	187.2 ± 25.2	164.7 ± 14.9	200.0 ± 44.9	140.3 ± 11.3	**196.0 ± 24.0b**
	HbA1c (%)	7.3 ± 0.3	7.3 ± 0.3	7.2 ± 0.5	7.4 ± 0.3	7.3 ± 0.3	7.5 ± 0.4	6.9 ± 0.5	7.5 ± 0.3
	TC (mg/dL)	225.2 ± 24.9	206.4 ± 15.4	211.5 ± 16.6	211.9 ± 18.7	210.7 ± 17.0	214.3 ± 17.2	182.1 ± 11.0	230.0 ± 18.2
	TG (mg/dL)	248.7 ± 108.4	189.6 ± 35.3	184.6 ± 27.0	219.9 ± 61.0	220.7 ± 53.1	171.0 ± 28.6	177.3 ± 22.3	224.5 ± 61.5
	HDL-C (mg/dL)	41.7 ± 7.7	38.4 ± 3.4	44.7 ± 2.9	36.0 ± 4.7	**35.6 ± 3.9 a**	48.7 ± 3.8	40.8 ± 3.7	38.4 ± 4.7
	LDL-C (mg/dL)	135.2 ± 15.1	130.9 ± 12.3	133.9 ± 15.7	131.1 ± 12.6	130.6 ± 11.9	136.2 ± 16.7	108.4 ± 8.2	146.8 ± 13.3
	VLDL-C (mg/dL)	48.5 ± 22.0	35.7 ± 6.6	32.9 ± 3.5	43.3 ± 12.2	43.3 ± 10.6	29.6 ± 2.6	33.1 ± 3.1	43.3 ± 12.2
	BMI (kg/m^2)	25.9 ± 1.3	26.6 ± 0.5	26.8 ± 0.7	26.1 ± 0.7	26.2 ± 0.6	26.8 ± 1.1	26.7 ± 0.6	26.2 ± 0.7
	BSA (m^2)	1.8 ± 0.1	1.8 ± 0.04	1.8 ± 0.04	1.8 ± 0.05	1.8 ± 0.04	1.7 ± 0.1	1.8 ± 0.03	1.8 ± 0.1
	WC (cm)	100.8 ± 2.5	95.3 ± 4.8	95.8 ± 2.9	97.8 ± 5.4	95.3 ± 3.5	100.8 ± 8.5	90.0 ± 3.8	100.1 ± 4.3
Liver Function	ALT (U/L)	20.8 ± 2.2	20.8 ± 3.7	17.0 ± 4.0	22.4 ± 3.3	21.0 ± 3.2	20.0 ± 4.0	22.3 ± 2.3	20.3 ± 3.3
	AST (U/L)	17.3 ± 1.8	33.9 ± 15.9	46.8 ± 32.3	19.3 ± 1.8	35.0 ± 15.8	14.5 ± 0.9	48.6 ± 31.9	18.3 ± 1.9
	GGT (U/L)	20.1 ± 0.2	18.6 ± 4.2	20.0 ± 2.1	18.6 ± 4.2	18.3 ± 5.4	20.0 ± 0.5	14.0 ± 6.0	21.3 ± 4.2
	CRP (mg/dL)	0.6 ± 0.2	2.0 ± 0.4	0.4 ± 0.1	2.3 ± 0.4	2.2 ± 0.5	1.1 ± 0.3	2.1 ± 0.3	1.9 ± 0.5
Kidney Function	BUN (mg/dL)	15.7 ± 0.7	17.8 ± 2.4	17.9 ± 2.9	16.9 ± 2.3	16.6 ± 1.5	18.8 ± 5.9	16.8 ± 2.5	17.4 ± 2.3
	Uric acid (mg/dL)	5.9 ± 0.1	5.4 ± 0.5	5.2 ± 0.5	5.7 ± 0.5	6.1 ± 0.4	4.9 ± 0.4	5.2 ± 0.5	5.7 ± 0.5
	Urea (mg/dL)	35.50 ± 3.18	45.0 ± 8.4	54.3 ± 14.0	35.6 ± 5.3	42.6 ± 8.4	42.6 ± 10.2	52.8 ± 17.5	38.0 ± 5.1
	Creatinine (mg/dL)	0.8 ± 0.1	1.1 ± 0.2	1.3 ± 0.3	0.8 ± 0.07	1.1 ± 0.2	0.9 ± 0.1	1.2 ± 0.3	0.9 ± 0.1
	Malb (mg/L)	11.7 ± 5.5	41.7 ± 18.7	52.8 ± 43.5	24.9 ± 6.8	40.8 ± 18.9	14.4 ± 2.8	58.8 ± 32.1	16.6 ± 6.0
	CKD-EPI	101.2 ± 0.8	92.5 ± 4.7	90.7 ± 7.1	95.9 ± 4.9	90.9 ± 4.9	102.5 ± 3.3	97.2 ± 2.3	92.9 ± 5.4

The results are shown as mean ± SEM (standard error). SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, HbA1c: Hemoglobin A1c, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma glutamyl transferase, BMI: Body mass index, BSA: Body Surface Area, WC: Waist circumference (cm), CRP: C-reactive protein, BUN: Blood urea nitrogen, Malb: Microalbuminuria, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration formula value (mL/min/1.73 m^2).
Bold values indicate statistical significance. a, $p = 0.036$; b, $p = 0.05$.

Table 8
The effects of *Rev-erb beta* common genotypes and alleles on biochemical parameters in the T2DM patients with and without obesity.

Parameters		Rev-erb beta Chr3:24003765 exon 5 A > G				Rev-erb beta rs924403442 (Chr3:24006717) G > T			
		Obese		Non obese		Obese		Non obese	
		AA (n = 14)	AG + GG (n = 7)	AA (n = 16)	AG + GG (n = 5)	GG (n = 12)	GT + TT (n = 9)	GG (n = 9)	GT + TT (n = 12)
Demographic	SBP (mm Hg)	117.0 ± 5.0	138.0 ± 11.8	133.3 ± 11.7	135.0 ± 25.0	130.5 ± 15.1	142.0 ± 17.0	150.0 ± 15.3	124.0 ± 11.7
	DBP (mm Hg)	80.0 ± 2.0	82.4 ± 5.4	84.2 ± 3.7	75.0 ± 15.0	80.4 ± 5.3	84.0 ± 6.0	86.7 ± 3.3	79.0 ± 6.4
	Glucose (mg/dL)	151.7 ± 7.1	140.3 ± 16.5	172.6 ± 21.0	181.6 ± 18.5	140.6 ± 11.1	157.7 ± 6.9	164.6 ± 20.8	182.4 ± 24.6
	HbA1c (%)	7.8 ± 0.2	8.1 ± 0.3	7.3 ± 0.2	7.4 ± 0.7	7.8 ± 0.2	8.0 ± 0.2	7.4 ± 0.2	7.3 ± 0.4
	TC(mg/dL)	197.3 ± 14.1	185.3 ± 12.1	203.1 ± 13.6	239.4 ± 32.3	197.8 ± 12.7	187.3 ± 17.1	205.4 ± 20.8	216.6 ± 17.0
	TG (mg/dL)	155.4 ± 11.0	171.4 ± 27.3	186.0 ± 42.8	272.0 ± 89.4	171.4 ± 15.7	146.6 ± 16.1	228.6 ± 73.6	189.9 ± 41.8
	HDL-C (mg/dL)	43.6 ± 3.1	40.2 ± 3.1	41.4 ± 3.4	32.7 ± 7.7	41.0 ± 2.0	44.4 ± 4.8	37.9 ± 4.9	40.4 ± 4.4
	LDL-C (mg/dL)	125.5 ± 12.2	115.7 ± 10.8	125.0 ± 9.6	155.0 ± 25.3	126.7 ± 12.1	116.3 ± 13.2	124.3 ± 14.8	138.0 ± 12.8
	VLDL-C (mg/dL)	28.1 ± 2.2	28.2 ± 3.0	35.5 ± 8.4	51.7 ± 18.0	29.4 ± 2.2	26.5 ± 2.9	43.3 ± 14.3	36.5 ± 8.5
	BMI (kg/m2)	34.6 ± 1.0	34.8 ± 1.8	26.3 ± 0.6	26.6 ± 1.0	35.1 ± 1.1	34.0 ± 1.5	26.4 ± 0.9	26.4 ± 0.6
	BSA (m2)	1.9 ± 0.0	1.9 ± 0.1	1.8 ± 0.0	1.8 ± 0.1	1.9 ± 0.0	1.9 ± 0.0	**1.9 ± 0.1 a**	1.7 ± 0.0
	WC (cm)	109.8 ± 2.8	110.1 ± 2.8	96.1 ± 4.4	100.0 ± 1.5	110.8 ± 2.5	108.8 ± 3.6	94.7 ± 3.8	99.0 ± 5.6
Liver Function	ALT (U/L)	20.5 ± 2.1	25.7 ± 2.5	22.9 ± 3.0	13.7 ± 3.2	23.1 ± 2.6	21.3 ± 2.1	22.6 ± 5.7	19.6 ± 2.6
	AST (U/L)	18.2 ± 1.3	25.0 ± 5.3	31.6 ± 13.2	16.0 ± 2.4	21.4 ± 3.1	18.4 ± 1.5	41.4 ± 22.6	16.9 ± 1.3
	GGT (U/L)	21.8 ± 4.5	82.3 ± 54.8	16.0 ± 2.4	18.4 ± 1.5	69.8 ± 41.0	19.8 ± 3.9	15.7 ± 3.8	22.0 ± 5.9
	CRP (mg/dL)	4.0 ± 1.1	5.0 ± 2.6	2.3 ± 0.4	1.7 ± 0.3	4.6 ± 1.9	4.1 ± 1.4	1.9 ± 0.8	2.1 ± 0.5
Kidney Function	BUN (mg/dL)	15.0 ± 0.9	15.0 ± 2.8	17.2 ± 2.0	17.3 ± 5.0	15.1 ± 1.5	14.8 ± 1.4	17.7 ± 2.0	16.9 ± 2.8
	Uric acid (mg/dL)	5.8 ± 0.5	6.0 ± 1.0	5.6 ± 0.4	6.1 ± 0.4	5.4 ± 0.7	6.5 ± 0.6	5.3 ± 0.5	5.8 ± 0.6
	Urea (mg/dL)	33.6 ± 1.8	31.4 ± 4.3	43.3 ± 8.2	40.5 ± 8.3	32.7 ± 2.6	33.1 ± 2.7	50.8 ± 10.6	34.5 ± 6.5
	Creatinine (mg/dL)	0.8 ± 0.0	0.9 ± 0.1	1.0 ± 0.2	1.1 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	1.2 ± 0.3	0.9 ± 0.1
	Malb (mg/L)	36.7 ± 18.6	20.6 ± 8.6	20.8 ± 6.0	74.4 ± 55.2	22.6 ± 7.6	44.1 ± 29.5	23.4 ± 8.3	45.0 ± 28.2
	CKD-EPI	88.8 ± 3.1	71.0 ± 25.1	94.8 ± 3.7	92.1 ± 12.4	85.1 ± 7.1	82.2 ± 3.1	90.5 ± 5.3	98.3 ± 5.8

The results are shown as mean ± SEM (standard error). SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, HbA1c: Hemoglobin A1c, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol, BMI: Body mass index, BSA: Body Surface Area, WC: Waist circumference, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma glutamyl transferase, CRP: C-reactive protein, BUN: Blood urea nitrogen, Malb: Microalbuminuria, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration formula value (mL/min/1.73 m2).
Bold values indicate statistical significance. a, p = 0.49.

Rev-erb alpha intronic rs2314339 common G allele frequency was high in Spanish Mediterranean (n = 1404) and North American whites (n = 810). They showed that the frequency of the minor A allele (AA + AG) was lower in the abdominally obese group than in the non-abdominally obese group ($p < 0.05$), and that the A allele was associated with lower obesity parameters such as waist circumference, body weight, BMI ($p < 0.05$). In the same study, *Rev-erb alpha* rs2314339 A allele was not associated with BMI values in the Mediterranean population taking low monounsaturated fatty acids ($p > 0.05$), however, this allele was found to be related with lower BMI in individuals taking high levels of monounsaturated fatty acids ($p < 0.05$). Besides, it was reported that individuals carrying A allele showed more physical activity in both populations ($p < 0.05$). Researchers have suggested that the study results provide evidence of the relationship between circadian rhythm and obesity, considering the role of Rev-erb alpha in the control of the biological clock (Garaulet et al., 2014).

In the study by Dashti et al. including 28.190 European descent from the Cohorts for Research in Genomic Epidemiology (CHARGE) Consortium, the effect of circadian related genes on cardiometabolic properties was analyzed and found no correlation between *Rev-erb alpha* rs2314339 C > T variation and glycemic properties ($p > 0.05$) (Dashti et al., 2015). In the same study, each additional 1% total fat intake in the presence of *Rev-erb alpha* rs2314339 T allele was found to be associated with 0.0024 higher HOMA-IR (p = 0.04), and each additional 1% saturated fatty acid intake in the presence of *Rev-erb alpha* rs2314339 T allele was associated with a 0.005 kg/m^2 higher BMI ($p < 0.01$).

These studies mentioned above indicate that Rev-erb alpha may have a potential role in obesity and related disorders. In our study, serum HDL-C levels in T2DM patients were found to be significantly lower in patients with Rev-erb alpha rs2314339 C > T (G > A) homozygous common CC genotype than those with minor T allele ($p = 0.027$). *Rev-erb alpha* rs2314339 SNP is located very close to the beginning of exon 3, causes a cytidine (C) to Thymidine (T) change in the sequence and may affect RNA processing by altering specific short oligonucleotide sequences known as exonic splicing enhancer (ESE). ESEs serve as binding sites for serine/arginine-rich protein members involved in alternative splicing regulation (Meshorer et al., 2002; Campos-de-Sousa et al., 2010). In silico analysis showed that the rs2314339 T allele disrupted the ESE consensus motif for the splicing-associated SC35 protein (Campos-de-Sousa et al., 2010). Therefore, this variation may have a functional effect although it is in the intronic region. In our study, we also observed the relationship of other *Rev-erb alpha* intronic SNPs serum HDL-C levels. We also showed the relationship of other Rev-erb alpha SNPs with HDL levels. The minor A allele of *Rev-erb alpha* rs72836608 was associated with lower serum HDL-C levels in total T2DM group ($p < 0.05$). Also, the *obese* T2DM subjects with rs72836608 minor A allele showed a lower HDL-C level than those with the CC homozygote genotype, but the association was not statistically significant (p > 0.05). In addition, in *obese* T2DMs, *Rev-erb alpha* intronic rs2102928 was found to be significantly associated with low HDL levels. We suppose that the association of both *Rev-erb alpha* variants with HDL-C levels may be due to the repression of ApoA1 which is a component of HDL by Rev-erb alpha as reported by Vu-Dac et al. (1998) in rats, which needs to be confirmed in humans. In our study, decreased serum HDL-C levels in diabetic patients carrying Rev-erb alpha rs2314339 CC genotype support this hypothesis. In our study, unlike Garaulet et al. (2014), the distribution of *Rev-erb alpha* rs2314339 SNP was not different between the total T2DM patient and control groups, and between the obese and non-obese T2DM patient groups. The different results obtained from these studies may result from the studies being conducted in different ethnic groups and different diseases and/ or different size of the study groups. In our study, the effect of Rev-erb alpha rs2314339 SNP on BMI and other obesity parameters was not observed in both total T2DM patient group and obese and non-obese T2DM subgroups. In this respect, our findings differ from those of Garaulet et al. (2014). However, monounsature fatty acid consumption is lower in the Istanbul city, where our study is conducted, compared to those living in the Mediterranean regions (Mahley et al., 1995). Considering this feature of our study group, our finding is consistent with that of Garaulet et al., who reported that *Rev-erb alpha* rs2314339 SNP was not associated with obesity in those taking low amounts of monounsaturated fatty acids.

Our study demonstrates different effects of *Rev-erbs* polymorphisms in male and female patients. *Rev-erb alpha* rs72836608 (C > A) and rs2314339 (C > T) were associated with low HDL-C levels in male T2DM patients, and in the presence of a rare T allele of *Rev-erb alpha* rs2102928 (C > T). In female patients, CC genotype of *Rev-erb alpha* rs2102928 (C > T) was associated with high microalbuminuria levels. The GG genotype of Rev-erb beta rs9244403442 G > T was associated with low HDL and high body surface area (BSA) values in female patients, but this effect was not observed in male T2DMs. Also, Rev-erb beta Chr3: 24,003,765 G allele was associated with increased creatinine levels in female controls, and this relationship was not found in male controls. Our findings suggest that rev-erb polymorphisms have different effects by gender, confirming the study of Ruano et al. (2014). BSA is a value used to define body size, a biometric unit used to adjust mass and volume (Ristow et al., 2010), effective in determining body fat mass in obese and non-obese patients (Sardinha et al., 2006). According to this formula; 1.81 ± 0.19 m^2 was considered as normal weight, 1.97 ± 0.16 m^2 as overweight, and 2.14 ± 0.21 m^2 as obese cases (Du Bois and Du Bois, 1989). The mean values of BSA in our study were in the normal reference ranges in the patient and control groups, and no statistical significance was detected. However, *Rev-erb beta* Chr3:24003765 SNP in the control group and *Rev-erb beta* rs924403442 SNP in the T2DM group and also in the non-obese and female T2DM subgroups were associated with high BSA levels in a normal weight range ($p < 0.05$).

Loss of Rev-erb alpha and Rev-erb beta, which mediate the regulation of the core clock function and the interaction between circadian rhythm and metabolism, causes marked hepatosteatosis in mice (Bugge et al., 2012). Sun et al. reported that the *Rev-erb alpha* deletion in mice disrupts the circadian rhythm in the liver, increases hepatic lipogenesis and 2-fold increase in hepatic triglyceride (TG) content (Sun et al.2011). It has been suggested that there is a link between non-alcoholic fatty liver disease (NAFLD) and circadian rhythm, which is characterized by increased intrahepatic TG content (steatosis) and lipogenesis imbalance in the liver (Gnocchi et al., 2015). Elevated ALT (Neuschwander-Tetri et al., 2010; Anty et al., 2010) and TG levels (Simental-Mendía et al., 2012; Tomizawa et al., 2014) are clinically and histologically associated with NAFLD and are used in the diagnosis of NAFLD. Moreover, studies show that diabetic patients with NAFLD have higher ALT, AST, GGT levels and AST:ALT ratio compared to diabetic patients without NAFLD. In our study, levels of liver enzymes (ALT, AST, GGT) were not different between control and total T2DM groups and between *obese* and *non-obese* patients. *Rev-erb beta Chr3:*24003765 (A > G) and *Rev-erb beta* rs924403442 (G > T) was found to be associated with serum ALT levels in the healthy controls, while no significant effects of Rev-erb SNPs on serum ALT and AST enzyme levels were observed in both total T2DM group and *obese* and *non-obese* T2DM subgroups. However, *Rev-erb alpha* Chr17: 38,253,751 (T > C) C allele, rs72836608 (C > A) CC genotype, and rs2314339 (C > T) T allele and *Rev-erb beta* Chr3: 24,003,765 (A > G) rare G allele were associated with increased serum GGT levels in *obese* T2DM patients. In *non-obese* patients, *Rev-erbs* SNPs had no effect on serum GGT levels. Obesity, especially central obesity, has been identified to be strongly associated with increased serum GGT levels. It is suggested that high GGT levels are a marker for visceral fat, and especially for hepatic steatosis, which leads to hepatic insulin resistance (Perry et al., 1998). In this context, the effect of *Rev-erb alpha* and *Rev-erb beta* SNPs on increased GGT levels in only *obese* T2DMs both confirms the relationship between GGT-obesity-T2DM and also indicates the effect of

changes in *Rev-erb* genes on liver function in obese T2DMs.

Evidence for kidney function interaction of circadian rhythm and clock genes has been increasing in recent years (Hsu et al., 2012; Oishi et al., 2004). Su et al. have shown that in db/db mice, which are animal models for T2DM, blood pressure circadian rhythm is severely impaired (Su et al., 2012)., and in another study they reported that the Rev-erb alpha expression profile was suppressed relative to controls (Su et al., 2008). Albuminuria is a good predictor of poor renal function and cardiovascular disease in patients with T2DM (Basi et al., 2008). Microalbuminuria occurs due to increased albumin passage through the glomerular filtration barrier and is a risk factor for vascular disease and diabetic nephropathy. Glomerular endothelial dysfunction, particularly damage in the glycocalyx, constitutes the initial stage of diabetic microalbuminuria (Satchell and Tooke, 2008). In our study, microalbuminuria levels ($p < 0.05$) and systolic blood pressure ($p < 0.05$) were lower in T2DM patients with *Rev-erb alpha* intronic rs2102928 T allele than those with CC genotype. Moreover, it was found that the prevalence of nephropathy was higher in patients with Rev-erb alpha Chr17:38253751 rare C allele than patients with normal TT genotype close to statistical significance ($p = 0.056$). Diabetic nephropathy is characterized by microalbuminuria, diabetic glomerular lesions and decreased glomerular filtration rate in diabetic patients. Hemodynamic factors such as increased angiotensin-converting enzyme, endothelin-1, and urotensin II levels in diabetics; metabolic factors such as endogenous fructose production (Lanaspa et al., 2014), the formation of reactive oxygen species, accumulation of glycation end products; inflammation and growth factors have been implicated in the pathogenesis of diabetic nephropathy (Lim, 2014). Rev-erb alpha was shown to improve endothelin-1-induced cardiac hypertrophy in human cardiomyocytes (Zhang et al., 2017). Based on the increased prevalence of nephropathy in T2DM patients with *Rev-erb alpha* Chr17:38253751 rare C allele in our study, we can assume that Rev-erb alpha may have an effect on the expression of parameters involved in the pathogenesis of nephropathy such as endothelin-1.

Understanding the pathogenesis of type 2 diabetes is important for developing therapeutic approaches. Considering the association between *Rev-erb alpha* and *Rev-erb beta* gene variants with biochemical and clinical parameters in T2DM patients, Rev-erbs may be potential targets for the treatment of T2DM and further studies with larger study groups will be important for this purpose.

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CRediT authorship contribution statement

Bengu Tokat: Methodology, Investigation, Writing - original draft. **Deniz Kanca-Demirci:** Investigation. **Nurdan Gul:** Conceptualization, Data curation. **Ilhan Satman:** Supervision. **Oguz Ozturk:** Software, Validation. **Aclan Ozder:** Data curation, Formal analysis. **Ozlem Kucukhuseyin:** Validation, Visualization. **HulyaYilmaz-Aydogan:** Funding acquisition, Project administration, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2020.145058.

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