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Screening of *MC4R, LEP, LEPR, POMC, SH2B1*, and *SIM1* genes in Turkish children with severe early-onset obesity

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Abstract

The aim of this study was to determine the prevalence of leptin (LEP), leptin receptors (LEPR), melanocortin-4-receptor (MC4R), proopiomelanocortin (POMC), single-minded 1 (SIM1), and SH2B1 gene variations in Turkish children and adolescents, and to conduct a detailed examination of the clinical and laboratory findings of patients with variants. In this study, we included 49 children and adolescents (29 male/20 female) who presented to the Pediatric Endocrinology clinic of Erzurum Regional Training and Research Hospital between 2017 and 2020 with obesity. Family history with regards to obesity, parental consanguinity, obesity-related comorbidities, anthropometric measurements, and laboratory tests of the patients were recorded in the clinical evaluation. LEP, LEPR, MC4R, POMC, SIM1, and SH2B1 genes, which are associated with monogenic obesity, were evaluated by the next generation sequencing analysis in all patients. The mean age of 49 patients included in the study was 8.4 ± 5.2 years (range: 0.6–16.8), their mean height standard deviation score (SDS) was 0.9 ± 1.6, mean body mass index (BMI) was 31.3 ± 8.1 kg/m2, and their mean BMI SDS was 3.5 ± 0.6. A total of four different variants (c.380C>T and c.870delG variants in MC4R gene; c.2992A>C and c.448delA variants in LEPR gene) were detected in four patients. The determination of a molecular etiology in patients with monogenic obesity is important in view of the treatment options to be introduced in the near future (MC4R agonist) and for the family to receive appropriate genetic counseling. In this study, we evaluated the clinical and genetic findings of the patients with monogenic obesity in detail, and contributed the findings of the novel variants to the literature.

Keywords: Monogenic obesity, MC4R, LEPR, novel variant

Introduction

Obesity is a condition that affects 12% of the world population and causes life-threatening comorbidities such as cardiovascular disease, hypertension, type 2 diabetes mellitus, dyslipidemia, and cancer [1]. Obesity is a multifactorial disease in which both genetic and environmental factors play a role. The role of heredity in obesity has been reported to be between 40% and 70% [2]. In terms of genetic etiology, obesity can be classified into three main groups: 1) Monogenic obesity: The group associated with a defect of a gene in the leptin–melanocortin signaling pathway 2) Syndromic obesity: The group in which obesity is accompanied with neurodevelopmental anomalies and organ malformations 3) Polygenic obesity: The group that is formed with the cumulative effect of multiple genes affecting energy intake and consumption [3]. The majority of the patients with childhood obesity are found to have polygenic obesity. On the contrary, monogenic obesity is extremely rare and its prevalence varies in different ethnic groups; however, it has been reported that the prevalence of monogenic obesity does not exceed 7% [4].

The genes associated with the monogenic form of obesity are mostly located in the signaling pathway of hypothalamic leptin–melanocortin, and hyperphagia and severe early-onset obesity are observed in this type of genetic defect of the signaling pathway [5]. Leptin is a cytokine secreted from adipocytes, which is responsible for maintaining the energy balance in the hypothalamus and inducing a feeling of satiety. The leptin binds to leptin receptors (LEPR) on the cell surface of the neurons located in the arcuate nucleus region of the hypothalamus and activates the signaling pathway of leptin–melanocortin. This activation stimulates the proopiomelanocortin (POMC) neurons to release POMC, which is an anorexigenic peptide. POMC is transformed into α-, β-, and γ-melanocyte stimulating hormone (MSH) via post-translational processing, and α-MSH protein binds to the melanocortin-4 receptor (MC4R) on the cell surface of the neurons

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in the paraventricular nucleus to create an anorexigenic signal. Downstream molecules such as single-minded 1 (SIM1), brain-derived neurotrophic factor, and neurotrophic receptor tyrosine kinase 2 are involved in the transmission of the signal to the upper cortical centers after stimulation of the MC4R. It is also known that the molecule SH2B1 regulates energy balance and body weight by increasing the sensitivity of hypothalamic leptin [3].

The leptin is a protein at a size of 16 kD, encoded by the LEP gene located in chromosome 7q32.1. Homozygous variations in the LEP gene were associated with the phenotype "Obesity, morbid, due to leptin deficiency" (MIM: #614962) in the OMIM® database. So far, 4 variants in the LEP gene have been detected in the HGMD® Professional database (https://portal.biobase-international.com/hgmd/pro/all.php). Leptin receptor is a protein at a size of 132.4 kD, located in the chromosome 1p31.1 and encoded by the LEPR gene. The homozygous variations in the LEPR gene were associated with the phenotype "Obesity, morbid, due to leptin receptor deficiency" (MIM: #614963) in the OMIM® database. So far, 63 variants of the LEPR gene have been detected in the HGMD® Professional database (https://portal.biobase-international.com/hgmd/pro/all.php). The MC4R is a G-protein coupled 7 transmembrane receptor composed of 333 amino acids and is encoded by the MC4R gene located in the chromosome 18q21.32. So far, 186 variants in the MC4R gene have been detected in the HGMD® Professional database (https://portal.biobase-international.com/hgmd/pro/all.php). The MC4R gene has been most commonly associated with monogenic obesity, and its heterozygous or homozygous loss-of-function variants are known to cause severe early-onset obesity phenotype [6]. More rarely, variations in the POMC, SIM1, and SH2B1 genes have also been shown to cause early-onset obesity phenotype [7-9]. Currently, thanks to the development of next generation sequencing technologies, we are able to quickly and inexpensively analyze all the genes associated with disease with genetic heterogeneity. In addition, the use of therapeutic agents based on molecular etiology has been discussed within the scope of precision medicine applications. A metreleptin therapy is available for congenital leptin deficiency [10]. On the other hand, in the deficiency of POMC, LEPR, and MC4R, treatment with setmelanotide, a melanocortin-4 receptor agonist, which has passed phase 3, is being discussed [11,12]. Determination of the molecular etiology in patients with severe early-onset obesity will be more important in the future for determining specific treatment options. In this study, 49 patients with a preliminary diagnosis of nonsyndromic monogenic obesity were screened with the next regeneration sequencing method in terms of the six genes that were previously demonstrated to be associated with childhood obesity. The aim of this study was i) to determine the frequency of LEP, LEPR, MC4R, POMC, SIM1, and SH2B1 gene variations in Turkish children and adolescents and ii) to conduct a detailed examination of the clinical and laboratory findings of the patients with variants.

Material and Methods

Patients

This study included 49 children and adolescents (29 male/20 female) who presented to the Pediatric Endocrinology clinic of Erzurum Regional Training and Research Hospital with obesity between 2017 and 2020. Patients with the following criteria were included in the study: i) patients who started to gain weight before the age of 7 years, ii) body mass index standard deviation score (BMI SDS) >3. Patients with chronic diseases, patients with a history of use of medications that could cause weight gain, patients with an endocrine pathology that could cause obesity, and syndromic patients were not included in the study.

Clinical and Laboratory Examination

In the clinical evaluation, the patients' family history in terms of obesity, parental consanguinity, and obesity-related comorbidities were recorded. The height and body weight of the patients were measured by experienced nurses working in the pediatric endocrinology outpatient clinic. BMI was calculated in kg/m2. Body weight, height, and BMI SDS of all patients were evaluated using Turkish national anthropometric references [13]. Glucose, insulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), HbA1c, and serum lipids were measured in a blood sample taken after 12 hours of fasting. The diagnosis of dyslipidemia was made in accordance with the previously recommended criteria [14]. Insulin resistance was evaluated with the homeostasis model assessment-insulin resistance (HOMA-IR) data. HOMA-IR cut-off value was considered as 3.16 as recommended by previous studies in obese children [15-17]. Written informed consents were obtained from all participants. This study was approved by the Ethics Committee of Erzurum Regional Training and Research Hospital (Approval number: 2020/16-167).

Genetic Analysis

The genomic DNA was isolated from 400-µl of peripheral blood sample using QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. All exons and exon-intron boundaries of LEP (NM_000230.3), LEPR (NM_002303.6), MC4R (NM_005912.3), POMC (NM_000939.4), SIM1 (NM_005068.3), and SH2B1 (NM_001145795.1) genes were amplified via custom design primers. For amplification of the targeted regions, Thermo Fischer Scientific PCR kit (Lot No:1212311) was used (Thermo Fisher Scientific, Waltham, MA, USA). The primer sequences were summarised in Table 1. Amplified PCR products were visualised on gel electrophoresis (Figure 1) and sequenced on the Illumina MiSeq Platform using 500-cycle Reagent Kit V2. The raw data were analyzed through the Sophia DDM® data analysis platform. Alignment and variant detection were performed by Pepper®, a proprietary basic algorithm of Sophia Genetics, according to the human genome reference hg19. In population studies (Genome Aggregation Database, 1000 Genomes Project), variants with an allele frequency of less than 0.5% were evaluated. In silico prediction tools (SIFT, Mutation Taster, PolyPhen-2, Provean), VarSome, CADD, and GERP score were used to assess the pathogenicity of the novel variants [18]. The variants were categorized using American College of Medical Genetics and Genomics (ACMG) criteria [19].

Results

The mean age of 49 patients included in the study was 8.4 ± 5.2 years (between 0.6 and 16.8 years), mean height SDS was 0.9 ± 1.6, mean BMI was 31.3 ± 8.1 kg/m2, and mean BMI SDS was 3.5 ± 0.6 at presentation. Clinical, physical examination, and laboratory findings of all patients are given in Table 2.

Table 1. The primer sequences of *MC4R, LEP, LEPR, POMC, SH2B1*, and *SIM1* genes.

Gene	Exon	Forward primer	Reverse primer	Start Nucleotide Number of FP	End Nucleotide Number of RP	Product Size
MC4R	1	5'ATCAATTCAGGGGGACACTG-3'	5'GACAGCACTATCTGAGT-3'	1	507	507
MC4R	1	5'ATGCTCTCCAGTACCATAACA-3'	5'TGCAGAAGTACAATATTCAGG-3'	480	987	508
LEPR	2	5'-AGAAGGTTATGCAGCCATTCTACTATC-3'	5'-CCACAAAGGGACTGTGTTCATAAACTG-3'	110	560	450
LEPR	3	5'-TAAATTAAGAGACTTATCTATAATCCC-3'	5'-TAACTAGAAATAGGAAATTCTGTTAGC-3'	630	1078	449
LEPR	4	5'-CACATTGTACAATGGAAGCACAAAGTT-3'	5'-TGTTAAAATCATAGCCATAAGACATCT-3'	1100	1550	451
LEPR	5	5'-TTTTTTTTAATTCAGATGCAAACTGGA-3'	5'-TGCAAGAGGTTTATAATCTACTTTCCGT-3'	1640	2093	454
LEPR	6	5'-ACCCTTTAAGCTGGGTGTCCCAAATAG-3'	5'-AGCTAGCAAATATTTTTGTAAGCAATT-3'	2150	2591	442
LEPR	7	5'-TGACTTTATTTTATTCAGCTATAATTG-3'	5'-GTAATTGCTATGGGACTTAAGAGGGTC-3'	2640	3084	445
LEPR	8	5'-TTTTAGTAACGGTTCCACATCAACTTG-3'	5'-CAAGTTCAGGAAATATATTTCCCTCCC-3'	3150	3569	420
LEPR	9-10	5'-TCATTGAATTTTTTGGAGATTTTATGC-3'	5'-CAGGAAGCAAATAATCTGTAAGAC-3'	3740	4154	415
LEPR	11	5'-GACTGCTGTTTTAAACAACAAATCAG-3'	5'-CTGCATACAAATCTGCTAACACAAATG-3'	4200	4594	395
LEPR	12	5'-TGAAAATATAACACAATGTTTTTAGGC-3'	5'-TTTATGCCAATAAAATTAATCTAATGC-3'	4610	5014	405
LEPR	13-14	5'-GGTTTAAAATAAAATGTACTTCAGGGC-3'	5'-TGGACCATGAAGTCTTTTTAAGAGTA-3'	5100	5497	398
LEPR	15	5'-GAGACTGTGGCAGAGGCAAACTATATC-3'	5'-ATTGCAGGCTGCTTGAAAGATAATTTA-3'	5520	5916	397
LEPR	16	5′-TTCCCTTTAGTAGGTTATAAGTTCCTC-3'	5′-TTTTTGAAGTTTTCATTAACTGGCTAT-3′	5990	6390	401
LEPR	17	5′-TTTGGAAACTCCCTTGATAATTTAATC-3'	5′-CCTCACCATGAAAAATCTACAGAAGAC-3′	6410	6824	415
LEPR	18	5′-AGAGATTTGTGATGAATTCAGAAAATG-3′	5′-TTAAATCAGGGTTTGAATACGCGTAAG-3′	6900	7304	405
LEPR	19	5′-CGGAGGCATAGTTGATCTGGTGGCTAA-3′	5′-GCGCTTGAAATTTGTTTCTTCCTGATT-3′	7400	7802	403
LEPR	20	5′-CAAACTTCCATTTTCTGCCAGTATGAC-3′	5′-CATTGGTAGGCTTATGAAGGCTTTCAC-3′	7870	8271	402
LEP	1	5'-GCCAGAGCAGAAAGCAAA-3'	5'-TCAGGAGGCGTTCAATAA-3'	1	390	390
LEP	2	5'-GAGCACTTGTTCTCCCTCTT-3'	5'-TTCCCTTAACGTAGTCCTTG-3'	430	834	405
POMC	2	5'-CCATTCCCTCCTCCTTATCAC-3'	5'-TCACGCCTATCACTGGGAAT-3'	1	395	395
POMC	3	5'-CACCCGTCTGCCTTTCTTG-3'	5'-TGGCCCATGACGTACTTCC-3'	410	809	400
POMC	3	5'-GGCCGAGACTCCCATGTT-3'	5'-GCCAGTCAGCTCCCTCTTG-3'	860	1264	405
POMC	3	5'-GCCCAGTGAAGGTGTACCC-3'	5'-CCCTCACTCGCCCTTCTT-3'	1300	1709	410
POMC	3	5'-CCCCTGGTGACGCTGTTC-3'	5'-CGGCCATCCTAGAGGTGC-3'	1740	2159	420
SH2B1	1	5'-AGTAGGGTCGGACGTCTCTG-3'	5'-CCAACAAAAAGTGAGCGACA-3'	2200	2629	430
SH2B1	2	5'-GGCAGGACTGAGAAAGCAGT-3'	5'-TTTCCCTGTCTTCCGCTATC-3'	2650	3089	440
SH2B1	3-4	5'-ACCCGGCCTCCAGAGAG-3'	5'-TAGGGCATCTGGAAAACAGG-3'	3140	3594	455
SH2B1	5-7	5'-GGTAAAAGCATCAGGGGTCA-3'	5'-AGGCACAACAGCCTCCTCTA-3'	3605	4009	405
SH2B1	8-9	5'-ATTCCATCGGATCCTCTGTTC-3'	5'-CTATGGCCTCTTCCAATTCAA-3'	4050	4449	400
SIM1	1	5'-CAGCTGGCAGTAGAAGAAAAGG-3'	5'-CCAACCAAACATATCTGATGGA-3'	1	395	395
SIM1	2	5'-CACACTGGTGAACCTACATGAAA-3'	5'-CTCAGCCCAATGGAGACAAAT-3'	410	809	400
SIM1	3	5'-TTGTTTGTTTCAGTGGATCCTG-3'	5'-GCTTGACGTAGGGACTCAAAAA-3'	825	1229	405
SIM1	4-5	5'-TGCAATAGGTAGCAAGAAGCAG-3'	5'-TATTGGGACCCAGTCAGACTTT-3'	1250	1659	410
SIM1	6-7	5'-AGCACTGCTTTGGGGCTAAG-3'	5'-GTCGGAGAAGTCCCTCAGGA-3'	1680	2094	415
SIM1	8	5'-TGCTAATGGTGAAGACATCTGG-3'	5'-TGCCTCCGTACTTAGATCCTTT-3'	2150	2569	420
SIM1	9	5'-AACCACCCCACATAAGAAACCT-3'	5'-TTCTGACAACAACCAGACTCTCA-3'	2650	3054	405
SIM1	10	5'-TTTGTTTGTTTTCTCTTACTGATCG-3'	5'-GAAGATAACTATTTGGTCATTCACTCT-3'	3100	3509	410
SIM1	11	5'-ACATCATGTGAGCCTGTTTCAA-3'	5'-TGGAGAGACAGTAGGGTGGTCT-3'	3560	3964	405
SIM1	11	5'-ACTTCACCATGTGACCATATCC-3'	5'-CTGTGGCATAGTAAATGCTGGTAAT-3'	4050	4554	405

Table 2. Clinical and laboratory findings of the patients.

Features	Probands (n:49)
Mean ± SDS	29/20
Age (years)	8.4 ± 5.2
Height (cm)	130.8 ± 31.1
Height SDS	0.9 ± 1.6
Weight (kg)	61.4 ± 38.3
Weight SDS	3.8 ± 1.1
BMI (kg/m2)	31.3 ± 8.1
BMI SDS	3.5 ± 0.6
Consanguinity (yes/no)	7/42
Family history of obesity (yes/no)	29/20
Dyslipidemia (yes/no)	27/22
Diabetes mellitus (yes/no)	1/48
Hepatosteatosis (yes/no)	17/32
Glucose (mg/dL)	89.3 ± 7.3
Insulin (mIU/ml)	16.6 ± 10.3
HOMA-IR	3.6 ± 2.1
HbA1c (%)	5.4 ± 0.6
AST (IU/L)	25.6 ± 8.4
ALT (IU/L)	25.6 ± 12.3
Total cholesterol (mg/dL)	161.7 ± 32.8
Triglycerides (mg/dL)	159.1 ± 104.4
HDL (mg/dL)	41.2 ± 10.8
LDL (mg/dL)	103.4 ± 26.6

M: male, F: female, SDS standard deviation score, HDL: high density lipoprotein, LDL: low density lipoprotein, HOMA-IR: homeostasis model assessment-insulin resistance

A total of four different variants were detected in the MC4R and LEPR genes in four patients. In one patient, a heterozygous missense (NM_005912.3: c.380C>T p.S127L) variation was detected in the MC4R gene, which was previously reported in the literature. In another patient, a homozygous frameshift (NM_005912.3: c.870delG, p.I291Sfs*10) variant was detected in the MC4R gene, which was also previously reported in the literature. In one patient, a novel heterozygous missense (NM_002303.6: c.2992A>C, p.T998P) variation was found in the LEPR gene. A novel heterozygous frameshift (NM_002303.6: c.448delA p.N150Ifs*20) variation was found in the LEPR gene in another patient. Clinical and genetic findings of the patients with variants are summarized in Table 3.

Table 1. Clinical and genetic features of the patients with variants (+)

Patient	Sex/Age (years)	Birth weight (g)	Age at onset of obesity (years)	Height SDS/ BMI SDS	Additional findings	Family history	Laboratory findings (Fasting glucose mg/dL, insuline mI-U/L, HbA1c, HOMA-IR)	Mutant again	Variation type	MT	SIFT	PR	P	CADD	GERP	ACMG	gnomAD Exomes version 2.1.1 (allele frequency)	Novelty
1	M/15.2	2900	4	0.58/ 3.38	Hepatosteatosis Dyslipidemia	+	85/26.1/4.7/5.4	Het c.380C>T p.S127L	Missense	DC	T	N	PD	25.4	5.71	VUS	0.000171	-
2	F/2.2	3000	1	3.02/ 5.13	Hepatosteatosis Dyslipidemia	+	92/16.6/5.6/3.7	Hom c.870delG p.I291Sfs*10	Frameshift	DC	-	-	-	-	6.0599	LP	-	-
3	M/8.8	2900	3	1.76/ 3.02	Dyslipidemia	+	93/14/5.4/3.2	Het c.2992A>C p.T998P	Missense	P	D	N	PD	15.3	5.9299	VUS	-	+
4	F/1.5	3200	1	1.08/ 3.03	-	+	89/15/4.7/3.3	Het c.448delA p.N150Ifs*20	Frameshift	DC	-	-	-	-	5.3099	P	-	+

M: male, F: female, HOMA-IR: homeostasis model assessment-insulin resistance, Het: heterozygous, Hom: homozygous, MT: mutation taster, PR: Provean, P: PolyPhen2, CADD: Combined Annotation Dependent Depletion, GERP: Genomic Evolutionary Rate Profiling, gnomAD: Genome Aggregation Database, DC: disease causing T: tolerated, N: neutral, D: damaging, P: polymorphism, PD: probably damaging, VUS: variant of uncertain significance, LP: likely pathogenic P: pathogenic

Discussion

In this study, the LEP, LEPR, MC4R, POMC, SIM1, and SH2B1 genes, which have been most commonly associated with nonsyndromic obesity, were evaluated by the next generation sequencing method as targeted resequencing. There were four different variants detected in the MC4R and LEPR genes, out of which two variants were novel.

The MC4R gene has been the most frequently studied gene so far and has variants that have been found most commonly in patients with nonsyndromic obesity. In the studies with obese and adult patients, the prevalence of these variants has varied, but is usually in the range of 0.5%–8.6% [20-23]. In three different studies conducted in a Turkish pediatric population, the variant detection rates in the MC4R gene were 5.7%, 8.5%, and 8.6%, respectively [21-23]. In the present study, the rate of variant detection in the MC4R gene was determined to be 4%. It is considered that the different rates found in different studies in patients of the same ethnic origin may be due to the patient selection criteria.

The MC4R is a receptor located on the cell surface of the neurons in the paraventricular nucleus in the hypothalamus, forming an anorexigenic signal with α-MSH stimulus. Energy homeostasis is responsible for inducing the feelings of food intake and satiety. In the present study, we found a variant in the MC4R gene in two patients. The heterozygous missense variation (NM_005912.3: c.380C>T p.S127L) in Patient 1 was previously reported in the literature in two different families [24,25]. It has also been shown by a functional study that this variation affects receptor activity [24]. The homozygous frameshift (NM_005912.3: c.870delG, p.I291Sfs*10) variation that we detected in Patient 2 was previously reported as homozygous in one patient and heterozygous in another patient [22,23]. Our patient was diagnosed at the age of 2, similar to the patient with homozygous variation in the literature. In the patient of Aykut et al., BMI SDS was 7.3, but it was found to be 5.13 in our patient [23]. The patient in the study by Tunç et al. with the same variation as heterozygous was diagnosed at the age of 6, and the BMI SDS value was 3.07 [22]. It is well known that homozygous patients are more common in societies where consanguineous marriages are common and these patients have a more severe clinical presentation.

The LEPR is stimulated as a result of binding of the leptin (secreted from adipocytes) to the receptor, which ensures synthesis of α-MSH from POMC. The energy balance and a feeling of satiety are achieved through activation of the MC4R receptor by α-MSH. There are five defined isoforms of LEPR. In humans, isoform B is expressed in the hypothalamus at the highest rate. All isoforms contain an extracellular domain consisting of 839 amino acids. The extracellular domain consists of CRH1, Ig Domain, CRH2, and FNIII domains. The CRH2 domain constitutes the leptin-binding zone. The transmembrane domain consists of 21 amino acids, and there are intracellular domains of different sizes located more distally. It has been demonstrated that long and short intracellular domains are necessary for the interaction of LEPR with JAK-STAT proteins and the activation of this signaling pathway [26]. The novel heterozygous missense (NM_002303.6: c.2992A>C, p.T998P) variation found in Patient 3 was in the long intracellular domain of the LEPR protein, which has not yet been encountered in the population databases. The in silico analysis programs, such as SIFT and PolyPhen2, predict this variation as a disease causing. The variation has a CADD score of 15.3 and a GERP score of 5.92. Although no clear data can be revealed unless proven by functional studies, we predict that this rare variation may affect intracellular signal transmission. The novel heterozygous frameshift (NM_002303.6: c.448delA p.N150Ifs*20) variation found in Patient 4 is in the extracellular CRH1 domain of the LEPR protein. This variation leads to the formation of a premature stop codon by causing a shift in the open reading frame. It causes the LEPR protein consisting of 1165 amino acids to terminate at the 170th amino acid, resulting in the loss of CRH2 domain, which is the leptin-binding zone. It is known that biallelic variations in the LEPR gene lead to the phenotype of monogenic obesity. When the age at onset and severity of obesity were evaluated together, Patients 3 and 4 are consistent with the clinical presentations of patients with biallelic variation. In this case, there are two possibilities for both patients: i) there could be a variation in the unanalyzed promoter and untranslated regions of the LEPR gene ii) there could be heterozygous gross exonic deletions which is not detected by sequence analysis. In the literature, compound heterozygous cases with gross exonic deletions and point mutations have been reported. Therefore, promoter and untranslated areas of both the patients should be evaluated by the method of sequence analysis and by multiplex ligation-dependent probe amplification method for the detection of gross exonic deletions.

Out of the 49 patients included in this study, insulin resistance was found in 27 (55%) patients, diabetes mellitus in one patient, dyslipidemia in 27 (55%) patients, and hepatosteatosis in 17 (34%) patients. All patients with variants had insulin resistance, three patients were found to have dyslipidemia, and two patients were found to have hepatosteatosis. Hence, patients with nonsyndromic monogenic obesity should be monitored in terms of obesity-related comorbidities; diabetes mellitus, coronary artery disease, and dyslipidemia. Furthermore, increased physical activity and energy-restricted diet should be advised to these patients [27].

In conclusion, four different variants were found in two different genes (MC4R and LEPR) in a total of 49 patients with a diagnosis of nonsyndromic early-onset obesity. The variations observed in the MC4R gene were previously reported in the literature, whereas the variations detected in the LEPR gene were novel. Detection of molecular etiology in patients with monogenic obesity is important in view of the treatment options to be introduced in the near future (MC4R agonist) and for the family to receive appropriate genetic counseling. In this study, the clinical and genetic findings of the patients with monogenic obesity were evaluated in detail and the novel variants found were contributed to the literature.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
This study was approved by the Ethics Committee of Erzurum Regional Training and Research Hospital (Approval number: 2020/16-167).

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