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# *LEPR* Deficiency: Prevalence and Importance of a Novel Mutation and Significant Genetic Variants, Usually Underestimated

## Leptin Reseptör Eksikliğinde Tespit Edilen Yeni Bir Mutasyon ile Göz Ardı Edilen Genetik Varyantların Önemi ve Prevalansı

Atıl Bişgin

Department of Medical Genetics, Çukurova University Faculty of Medicine, Balcali Clinics and Hospital, Çukurova University AGENTEM (Adana Genetic Diseases Diagnosis and Treatment Center), Adana, Turkey

## Abstract

**Objevtice:** Diagnostic testing for leptin receptor deficiency, a rare cause of obesity, should be performed in cases where it may affect the clinical management. Therefore, molecular tests are required to grant a conclusive diagnosis. In this study, the clinical utility of molecular testing and the importance of genetic counselling resulting from all the genetic variants, including both, disease-causing mutations and polymorphisms has been outlined.

**Material and Methods:** The study consisted of samples of leukocyte-DNA in sixteen clinically deficient patients of leptin receptor. In order to identify the molecular basis, the *LEPR* gene sequencing was employed using next-generation sequencing platform (MiSeq System, Illumina) for all the exons, introns and exon-intron binding regions. In-silico analyses for novel mutations were carried out using SIFT, Polyphen2 and Mutation Taster. Paternal carrier testing was also accomplished.

**Results:** The causative mutation was identified in three out of sixteen patients with leptin receptor deficiency (18.75%). All these three patients carried the same, novel, homozygous p.P639L (c.1916C>T) mutation. Most interestingly, 62.5% of the patients (n=10) were found to be carrying at least one of the possible disease-risk-polymorphisms related to obesity, increased body mass index, insulin resistance and glucose intolerance.

**Conclusion:** This study presented with two important outcomes. First, the novel p.P639L mutation could be identified in three different patients and, second, but most important, the fact that polymorphisms of the leptin receptor gene, usually underestimated, is the main genetic predisposition factor for the Turkish population. It is, therefore, critical to identify not only the mutations but all the genetic variants responsible for leptin receptor deficiency, to aid in diagnosis, prevention, prognosis, treatment, and research.

**Keywords:** *LEPR* deficiency; morbid obesity; genetic testing; *LEPR* novel mutation

## Özet

**Amaç:** Leptin reseptör eksikliği, nadir bir obezite sebebi olup, tanı testlerinin yapılması klinik sağaltım açısından önemlidir. Dolayısıyla, kesin tanı için moleküler testler gereklidir. Bu çalışmada, sadece hastalık etkeni olan patojenik varyantlar değil, polimorfizmler de dâhil tüm genetik varyantların saptanmasının genetik danışmanlık ve hastalığın moleküler tanısı için klinik kullanılabilirliği vurgulanmaktadır.

**Gereç ve Yöntemler:** Çalışmada klinik olarak leptin reseptör eksikliği olan 16 hastaya ait, lökositten izole edilmiş DNA örnekleri kullanılmıştır. Hastalığın moleküler tanısı için *LEPR* geninin tüm ekzon, intron ve ekzon-intron bağlantı bölgeleri yeni nesil dizileme teknolojisi (MiSeq System, Illumina) kullanılarak sekanslanmıştır. Saptanan yeni mutasyonların in-siliko analizleri SIFT, Polyphen2 ve MutationTaster kullanılarak gerçekleştirilmiştir. Aile taraması ile taşıyıcılıklar tespit edilmiştir.

**Bulgular:** Çalışmaya alınan 16 leptin reseptör eksikliği hastasının 3 (%18,75)'ünde hastalık etkeni bir mutasyon saptanmıştır. Bu hastalarda saptanan varyant aynı olup; yeni, homozigot p.P639L (c.1916C>T) mutasyonudur. İlginç olarak, hastaların %62,5'inde (n=10) hastalığa yatkınlıkla ilişkili en az bir polimorfizm saptanmış olup; bunlar obezite, artmış beden kitle indeksi, insülin rezistansı ve glukoz intoleransı ilişkili varyantlardır.

**Sonuç:** Bu çalışmanın iki ana sonucu bulunmaktadır. Birincisi, üç ayrı hastada aynı ve yeni homozigot p.P639L mutasyonu bildirilmekte, daha da önemlisi ve ikincisi de leptin reseptör geninde göz ardı edilen polimorfizmlerin Türk populasyonu açısından hastalığın kliniği ve hastalığa yatkınlıkla olan ilişkisinin ortaya konmasıdır. Sonuç olarak, tanıda sadece mutasyonların değil, tüm genetik varyantların saptanması ve yorumlanması tanı, korunma, prognoz, tedavi ve araştırmalar açısından önemlidir.

**Anahtar kelimeler:** *LEPR* eksikliği; morbid obezite; genetik test; *LEPR* yeni mutasyon

**Address for Correspondence:** Atıl Bişgin, Çukurova University Faculty of Medicine, Department of Medical Genetics, Balcalı Clinics and Hospital, Çukurova University AGENTEM (Adana Genetic Diseases Diagnosis and Treatment Center), Adana, Turkey
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## Introduction

Leptin receptor (LEPR) deficiency is a rare cause of obesıty. LEPR deficiency causes the clinical effects to begin in the first few months of life (1). Though most of the affected patients lie in the normal range of birth weight, yet, they start gaining weight quickly due to the constant hunger. Right from the early childhood, patients have hyperphagia and develop their own abnormal eating behaviors that may include fighting for the food or even eating secretly. Nevertheless, the most important clinical outcome of these patients is the effect on sexual development. LEPR deficient patients have hypogonadotropic hypogonadism which may result in infertility (1-3).

LEPR deficiency is caused by mutations in *LEPR* gene that encodes the leptin receptor protein on the surface of many cells but is expressed mostly in the hypothalamus (2, 4). It is also one of the rare diseases with an autosomal recessive inheritance pattern, affecting the morbid obesity group (2). Mutations in *LEPR* gene causes a deficiency in the leptin receptors or an in-effective receptor complex. Consequently, this loss of function prevents the receptor from responding to leptin released from the fat cells. This triggers the activation of a series of signaling pathways, leading to excessive hunger, weight gain and decreased sexual development (4-9). However, the mechanism of these effects is not yet completely understood.

This inherited obesity resulting from LEPR deficiency is difficult to diagnose, due to considerable clinical overlap and since most patients typically do not show signs and symptoms of the condition (10, 11). Hence, molecular tests are required to obtain a conclusive diagnosis as well as to have the benefits of diagnosis including the pre-symptomatic diagnosis and screening, prevention, treatment, prognosis, and research.

Genetic counselling, together with clinical evaluation, plays a critical role in the appropriate use of molecular tests. In this study, the clinical utility of molecular testing and the importance of polymorphisms that are mostly underestimated by many laboratories and clinicians have been outlined by the authors using the experiences they have gained.

## Material and Methods

This study was carried out on the biological samples collected from The Cellular and Tissue Biobank of Cukurova University, Balcali Hospital and Clinics that focuses on rare diseases. A total of, sixteen pediatric patients (mean age of 21±1 months) with *LEPR* deficiency were referred to AGENTEM (Adana Genetic Diseases Diagnosis and Treatment Center), Cukurova University for molecular studies, and genetic counseling.

In order to identify the molecular basis, the genetic analysis including leptin receptor deficiency related *LEPR* gene was employed as a molecular diagnostics tool by using next-generation sequencing platform (MiSeq System, Illumina). The coverage of the test included all exons for each gene, at least 50 nucleotides upstream and downstream of each exon and 1 kb of both the 5’ promoter regions and the 3’ UTRs. Sequencing was performed on the leukocyte DNA collected from 16 patients with LEPR deficiency.

In-silico analysis for the novel mutations was carried out using SIFT, PolyPhen2 and Mutation Taster. All changes considered to have potential clinical relevance were confirmed by paternal testing to identify the carrier status.

ClinCalc (http://clincalc.com) was used to determine the post-hoc power of the study and to apply the Bonferroni correction. All the statistical analyses were performed using the GraphPad Prism software (GraphPad Software, Inc. USA), while the statistical significance was defined at $p \leq 0.05$. Hardy-Weinberg equilibrium analysis was performed for each polymorphism identified. A modified version of the human genome (www.varsome.com) was used as the major allele population-specific reference. Confidence interval (CI) as 95% was used to estimate the precision of the odds ratio. The chi-square test was also used to test the frequencies of the alleles and genotypes.

All the procedures performed in this study were in accordance with the ethical standards of the institutional ethical and national research committee and the Helsinki declaration. Informed consent of the patients and their families were conducted to keep all the information confidential was taken before the study began.

## Results and Discussion

All genetic variants detected in the patients are listed in Table 1 within the column homozygosity status.

The causative mutations were identified in three out of the sixteen patients (Patient 1, 2 and 3; sum up 18.75%) affected with LEPR deficiency and there was a novel homozygous p.P639L (c.1916C>T) mutation, in all these three patients, as shown in Figure 1. Neither of these patients had any consanguinity nor did they belong to the same city, but all had a middle-eastern origin. The in-silico analysis revealed that this variant was pathogenic and the mutation was a disease-causing one. Thus, paternal studies were performed and it was found that the mothers and fathers of all these three patients were first degree cousins and were heterozygous for the p.P639L (c.1916C>T) mutation in *LEPR* gene which confirms the results. This might have occurred because of the probability of carrying this mutation at a high frequency that can be speculated as a founder mutation in *LEPR* gene. However, further population studies must be carried out to support this conclusion.

The other significant data from this study included the frequency of polymorphisms that are mostly underestimated and usually left unreported in the genetic test reports. Ten out of the sixteen patients (62.5%) had at least one or more polymorphisms, as given in Table 1.

Among all the polymorphisms detected (n=10) in patients with LEPR deficiency, p.Q223R (c.668A>G) was the most common. Six out of these ten patients (60%) also showed other polymorphisms along with p.Q223R (c.668A>G).

Patients 4, 5 and 6 had two homozygote polymorphisms of p.K109R (c.326A>G) and p.Q223R (c.668A>G). The frequency of these alterations in heterozygous population is high, around 40% (3, 12). However, there are also studies indicating that homozygous p.K109R polymorphism is related to increased birth weight and body-mass index (2, 10, 13). The homozygous p.Q223R variant was found to be significantly associated with obesity in previous studies (14). A study also suggests that when these two variants occur together, they are also associated with glucose intolerance (15, 16).

Table 1. The genetic variants detected in the patients were classified according to the homozygosity and heterozygosity status regardless of pathogenicity.

| | Variants in LEPR Gene | | | |
|---|---|---|---|---|
| **Patient Number** | **p.P639L (c.1916C>T)** | **p.Q223R (c.668A>G)** | **p.K109R (c.326A>G)** | **p.S343S (c.1029T>C)** |
| 1 | Homozygote | - | - | - |
| 2 | Homozygote | - | - | - |
| 3 | Homozygote | - | - | - |
| 4 | - | Homozygote | Homozygote | - |
| 5 | - | Homozygote | Homozygote | - |
| 6 | - | Homozygote | Homozygote | - |
| 7 | - | Homozygote | Heterozygote | Heterozygote |
| 8 | - | Heterozygote | Heterozygote | - |
| 9 | - | Heterozygote | Heterozygote | - |
| 10 | - | Heterozygote | - | - |
| 11 | - | Heterozygote | - | - |
| 12 | - | Heterozygote | - | - |
| 13 | - | Heterozygote | - | - |
| 14 | - | - | - | - |
| 15 | - | - | - | - |
| 16 | - | - | - | - |

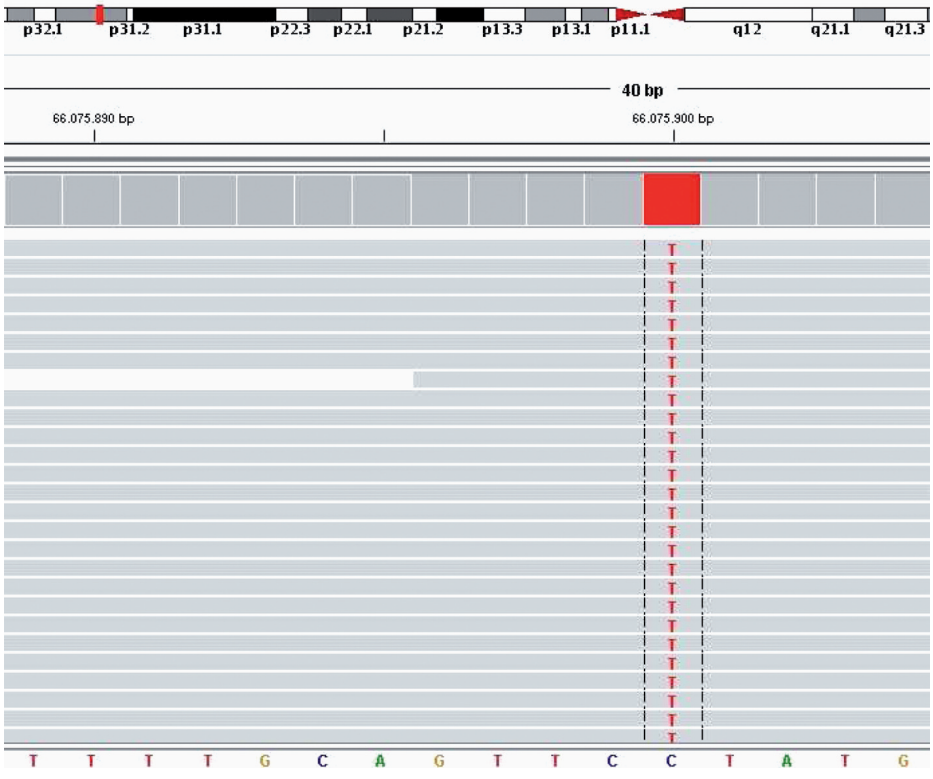


**Figure 1:** NGS data electropherogram tracing of *LEPR* gene, showing the novel homozygous p.P639L (c.1916C>T) mutation (patient 1 data is given, as an example).

Patient 7 had three heterozygous polymorphisms of p.K109R (c.326A>G), p.Q223R (c.668A>G) and p.S343S (c.1029T>C). p.S343S (c.1029T>C) is a synonymous variant that does not have any effect on the protein level. Since all these three variants with high population frequency (12) were heterozygous in this patient, no genetic effect was expected in the patient apart from only mild clinical findings, in case of any cumulative effect at all.

Patients 8 and 9 had two heterozygote polymorphisms of p.K109R (c.326A>G) and p.Q223R (c.668A>G). While patients 10 and 11 exhibited only p.Q223R (c.668A>G) heterozygous polymorphism, patients 12 and 13 showed only a single heterozygosity for p.K109R (c.326A>G) polymorphism in *LEPR* gene. The last three patients (patient numbers 14, 15 and 16) had a normal sequencing data. It is therefore important to carry out MLPA (multiplex ligation-depending probe amplification) for the deletion and duplication analysis of *LEPR* gene. Unfortunately, no MLPA probe has been designed for in-vitro diagnosis.

However, the undiagnosed LEPR deficiency patients in whom, genetic testing was not helpful in identifying the disease, might have had yet uncharacterized mutations in other genes.

For each of the 16 patients tested, at least one variant detected in 81.25% of the patients (n=13). In-silico analysis and family studies were performed to help define the pathogenicity of the novel changes. In addition to the polymorphisms, the causative novel mutation was identified in three out of sixteen patients. More interestingly, the identified alterations that are classified as polymorphisms, may have had an effect on the clinical findings, as per the literature review and data from the present study (16). The data in this, therefore, proposes a greater effort in the molecular diagnosis of rare diseases with appropriate genetic reporting in favor of diagnosis and prevention, and not just the control of co-morbidities, with the aim of enhancing the prognosis and a closer follow-up for the patients in order to improve their quality of life.

## Conclusion

To summarize, the availability of molecular genetic testing has profound implications for the clinicians, patients and their families. The benefits of genetic testing can be utilized for the diagnoses including the presymptomatic diagnosis and screening, prevention, treatment, prognosis, and research. However, a challenge still exists in the field of genomics that when potential novel genes/mutations/polymorphisms or phenotypes are detected, large group studies, functional studies, and model systems are needed. At the last place, even if a disease is monogenetic as in case of LEPR deficiency, results will still outcome as non-diagnostic or only about susceptibility due to possible defects on other genes or the epigenetic factors such as DNA methylation and histone modification, or epistasis.

In this study, a novel mutation, which is a disease-causing mutation, was identified in three different patients. The compound homo/heterozygosity for the polymorphisms was also determined that has helped in a better understanding of its association with obesity, increased body mass index, insulin resistance, and the glucose intolerance. While allelic heterogeneity can result in varying phenotypic severity, phenotypically identical disorders can also have an entirely different genetic basis (genocopy). It is, therefore, critical to identify the mutation/polymorphism-associated risks by molecular analysis. It is also important not to underestimate the disease associated polymorphisms that has to be specified in genetic testing reports.

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## Authorship Contributions

This study is entirely author's own work and no other author contribution.

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