A Novel Homozygous Frameshift Mutation in Exon 2 of *LEP* Gene Associated with Severe Obesity: A Case Report

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ABSTRACT

BACKGROUND: Monogenic obesity is a rare type of obesity caused by a mutation in a single gene. Patients with monogenic obesity may develop early onset of obesity and severe metabolic abnormalities.

CASE PRESENTATION: A two-and-half-year-old girl was presented to our clinic because of excessive weight gain and hyperphagia. She was born at full term, by normal vaginal delivery with birth weight of 2.82 kg and no complications during pregnancy. The patient was the second child of two healthy, non-obese Saudis with known consanguinity. She gained weight rapidly leading to obesity at the age of three months.

METHODS: The demographic data and clinical features were recorded. Blood samples were collected and tested for endocrine and metabolic characteristics and genetic studies. Mutations of the *LEP* gene were screened. The coding exons 2 and 3 and the corresponding exon–intron boundaries were amplified by polymerase chain reaction using specific primers, analyzed by direct sequencing using an ABI sequencer 3500 xL GA (Applied Biosystems), and evaluated using the JSI SeqPilot software. The resulting sequence data were compared with the reference MM_0002302.

CONCLUSION: We report a novel homozygous frameshift mutation c.144delin TAC (G1n49Thrfs*23) in exon 2 of the *LEP* gene associated with extreme obesity.

KEYWORDS: case report, *LEP*, mutation, obesity, genetics

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Background

Monogenic forms of childhood obesity are very rare; they are caused by a mutation in a single gene and are not affected by environmental factors.[1] To understand the mechanisms regulating energy intake and fat accumulation in the body, it is important to study the genetic alterations causing monogenic obesity. Mutations in only a few genes, *proopiomelanocortin (POMC)*, *leptin receptor (LEPR)*, *leptin (LEP)*, *proconvertase 1 (PC1)*, and *melanocortin* 4 *receptor (MC4R)*, are known to cause the development of severe obesity in early childhood.[1,2] Most of these genes are involved in the central nervous regulation of hunger and satiety, where the leptin/leptin receptor system plays a pivotal role.[3] Of all monogenic forms of obesity, the only one causally treatable is congenital leptin deficiency caused by homozygous mutations of the leptin gene.[4]

Human *LEP* is located on chromosome 7q31.3, and its translational product is leptin, which plays a decisive role in the regulation of human appetite; defects in *LEP* result in severe metabolic disorders.[5,6] Leptin is a hormone produced mainly by white adipose tissue, with multiple actions in the endocrine and immune systems, including glucose homeostasis, reproduction, bone formation, tissue remodeling, and inflammation.[7] It is a key regulator of energy homeostasis, by regulating energy intake and expenditure through its actions on the arcuate nucleus of the hypothalamus.[8,9]

In the clinical histories of patients with leptin deficiency, early development is usually normal. The most notable feature is intense hyperphagia with food-seeking behavior and aggressive behavior when food is denied. In the research setting, measurements of energy intake at ad libitum test meals reveal the extent of hyperphagia, with food intake three to five times than that of children of the same age as well as more hunger and impaired satiety seen after meals of fixed quantity and composition.[10] Current clinical recommendations suggest that children with a normal birth weight but rapid weight gain in the first few months of life leading to extreme obesity should be tested for congenital leptin deficiency.[11–13] Congenital leptin deficiency caused by homozygous mutations in the leptin gene results in impaired satiety, intense hyperphagia, and extreme early-onset obesity accompanied by multiple metabolic, hormonal, and immunological abnormalities. Children with leptin deficiency have marked abnormalities of T-cell

number and function,[10] resulting in high rates of childhood infection and therefore a high rate of childhood mortality.[14] In those who survive, obesity continues in adult life, with hepatic steatosis and hyperinsulinemia consistent with the severity of obesity.[15] In this manuscript, we have summarized the presentation of a Saudi child with a progressive increase in weight and hyperphagia.

Methods

This study was conducted in accordance with the Declaration of Helsinki and approved by the research and ethical committee of Prince Sultan Military Medical City (PSMMC), Riyadh, Kingdom of Saudi Arabia. Written informed consent was obtained from the parent of the patient. The demographic data and clinical features were recorded. Blood samples were collected for endocrinologic and metabolic testing and genetic studies.

Sequence analysis. Genomic DNA was extracted from a blood sample. The primers for *LEP* were designed with the software Primer Premier 5. Detailed information of the primers and the products is shown in Table 1. Genomic DNA was screened for mutations in the *LEP* gene (OMIM 164160) on chromosome 7q32.1. Coding exons 2 and 3 and the corresponding exon–intron boundaries were amplified by polymerase chain reaction (PCR) using the mentioned sets of primers and analyzed by direct sequencing. Direct Sanger sequencing was performed using an ABI sequencer 3500 xL GA (Applied Biosystems). The sequence data were evaluated using JSI SeqPilot software. The resulting sequence data were compared with the reference MM_0002302.

Results

Case presentation. A two-and-half-year-old girl was presented to our clinic because of excessive weight gain. She was born at full term, by normal vaginal delivery with a birth weight of 2.82 kg and no complications during pregnancy. Her history was significant for bronchial asthma treated with fluticasone inhaler 50 µg twice daily with no other medication. There was no history of excessive hair growth or skin rash, constipation, lethargy or decreased activity, abnormal movement, vomiting, irritability or loss of consciousness, or obstructive sleep apnea. Her family history included obesity on the paternal side. The patient is the second child of two healthy, non-obese Saudis with known consanguinity. The patient was born with normal size and weight but gained weight rapidly thereafter, leading to obesity at the age of three months. Parents are first-degree relatives (cousins) and they have another child, a normal baby boy.

The anthropometric and endocrinologic characteristics of the patient are summarized in Table 2. Her growth parameters were 71 cm height and 15.16 kg weight at the age of 11 months and 99 cm height and 33 kg weight at the age of 2.5 years. Her clinical examination was normal. She had hyperphagia and showed aggressive behavior for demanding food. The level of leptin was very low (<0.7 ng/mL). Clinical analysis showed normal levels of free thyroxine (T4), thyroid-stimulating hormone (TSH), cortisol, insulin, C-peptide, blood sugar, HbA1C, follicle-stimulating hormone, and luteinizing hormone. Her lipid profile was also normal.

Genetics. Sequencing revealed a homozygous deletion of one nucleotide and insertion of three nucleotides on position c.1444 in exon 2 of the *LEP* gene (c.144delins TAC), which led to a frameshift and a premature termination codon (Gln49Thrfs*23; Fig. 1). The result was confirmed by sequencing of an independent PCR product.

Discussion

Mutations in only a few genes are known to cause the development of severe obesity in early childhood.[1] Of all monogenic

Table 1. Details of primers used for amplification of exon 2 and 3 of *LEP*.

PRIMER	SEQUENCE	ANNEALING TEMPERATURE	PRODUCT SIZE (BP)
Exon 2 Forward	GTC TGG TAA TGT GGT TGG TAA T		
Reverse	TTC AGG AGG CGT TCA ATA AAT G	58 °C	364
Exon 3 Forward	CTG AGC CAA AGT GGT GAG G		
Reverse	GTG TCC ATG CAA TGC TCT TC	58 °C	591

Table 2. Anthropometric, endocrinological, and metabolic characteristics of the patient.

VARIABLES			REFERENCE RANGE
AGE	11 MONTHS	2½ YEARS	
Weight (kg)	15.16	33	
Height (cm)	71	99	
BMI (kg/m^2)	>30	33.6	
Leptin (ng/ml)	0.7	ND	2.0–5.6
Adiponectin (mg/ml)	6.0	6.5	5.0–7.5
Total cholesterols mg/dL	172	171	<170
Triglycerides mg/dL	155	152	<150
LH IU/L	0.1	0.5	0.1–3.3
AST U/L	15	20	10–35
ALT U/L	26	27	<29
T4 pmol/L	13.3	15.4	12–22
TSH uU/ml	2.31	3.01	0.27–4.20
FSH IU/L	2.4	2.84	1.0–3.25
Cortisol nmol/L	324	500	193–690
Insulin µIU/ml	8.3	34.1	2.6–40
C-peptide pmol/L	734	1520	366–1466
HbA1C	5.0	5.2	
Blood sugar mmol/l	4	5	

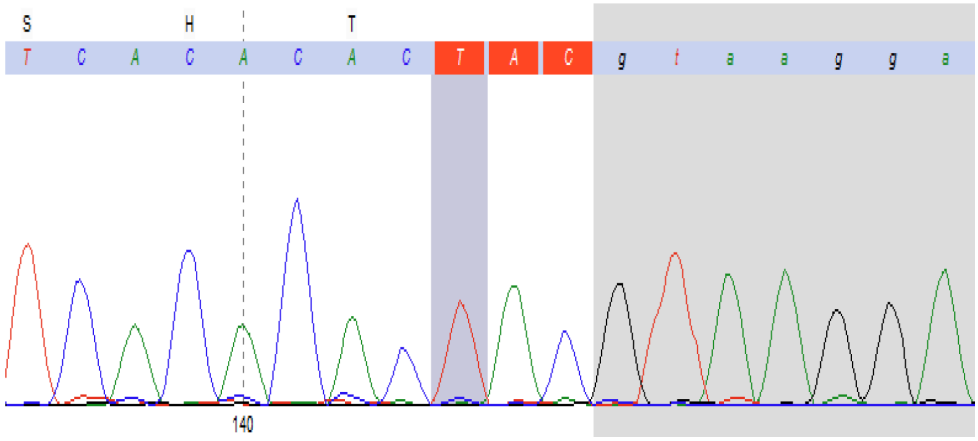

Figure 1. A homozygous deletion of one nucleotide and insertion of three nucleotides was detected on position c.1444 in exon 2 of the *LEP* gene (c.144delins TAC) which leads to a frameshift and a premature termination codon (Gln49Thrfs*23).

forms of obesity, the only one causally treatable is congenital leptin deficiency caused by homozygous mutations of the leptin gene.[4] Mutations in *LEP* genes are autosomal recessively inherited.[16] In this patient, a homozygous deletion of one nucleotide and insertion of three nucleotides were detected on position c.1444 in exon 2 of the *LEP* gene (c.144delins TAC), which led to a frameshift and a premature termination codon (Gln49Thrfs*23). Eight mutations in the *LEP* gene have been reported to be associated with congenital leptin deficiency in human beings.[13] To the best of our knowledge, the mutation seen in our patient has not been described in the literature or databases so far. This frameshift mutation probably results in degradation of the mRNA (nonsense-mediated decay) or in a truncation of LEP protein. Mutations in the gene encoding *LEP* typically lead to an absence of or decrease in circulating leptin and to extreme obesity. This form of leptin deficiency caused by mutations in the leptin gene is very rare. A review by Funcke et al.[13] found that a total of 30 patients had been reported to carry mutations in the *LEP* gene, and most of the patients described had consanguine parents. *LEP* mutation associated with congenital obesity has been identified and reported in patients of Pakistani,[10,17–20] Turkish,[21–24] Egyptian,[25] Indian,[26] Turkmenistani,[27] and Austrian[28,29] backgrounds. The evaluation of such patients before and during leptin replacement therapy has unveiled the importance of leptin in the homeostasis of several systems, such as the brain, immunity, and glucose metabolism. It is plausible that mutations in the *LEP* gene could result in a bioinactive form of the hormone in the presence of apparently appropriate leptin levels.[30]

Body composition measurements have shown that these disorders are characterized by the preferential deposition of fat mass. The mean percentage body fat among homozygous carriers of *LEP* mutations has been reported as very high at 58%, compared with 45% for equally obese children of the same age.[31] *LEP* is expressed in adipose tissue, and its product leptin affects food intake and energy expenditure. Therefore, mutations in *LEP* can possibly damage the function of leptin and disturb the metabolic balance, leading to severe obesity and other metabolic disorders. Mechanistically, defects in the synthesis and/or secretion of the hormone have been proposed and demonstrated for some of these mutations. Affected patients can be successfully treated with recombinant human leptin.[13] Moreover, leptin replacement therapy has been reported to reverse endocrine and metabolic alterations associated with leptin deficiency.[32]

Leptin deficiency has also been associated with hypothalamic hypothyroidism characterized by a low free T4 and high serum levels of bioinactive TSH.[10] However, in this patient, levels of T4 and TSH were within normal range, indicating normal thyroid function.

There are several limitations of this study. First, screening for the mutation in the patient's parents and sibling could not be performed. Second, the frequency of this mutation in the normal Saudi population is also not known. In addition, more clinical data regarding visceral and subcutaneous fat would be of great help in ascertaining some of the conclusions. Finally, functional study of this novel mutation was not carried out to elucidate the mechanism of the disease.

Conclusion

The diagnosis of obesity caused by leptin deficiency is suggested. A novel homozygous frameshift mutation c.144delin TAC (G1n49Thrfs*23) in exon 2 of the *LEP* gene was detected. We speculated this mutation to be a casual mutation leading to monogenic obesity in this Saudi child. However, functional studies should be performed to elucidate the substantial mechanism. We advised the counseling of the parents for starting leptin replacement therapy and monitoring its outcome.

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Ethical Approval and Consent to Participate

This study was approved by the research and ethical committee of PSMMC, Riyadh, Kingdom of Saudi Arabia, and written informed consent was obtained from the parent of the child before recruitment. The parent also gave consent for publication of this report.

Availability of Data and Material

All data related to this study, including information about the patient and mutation analysis, are available in the Research Center, PSMMC, Riyadh, Kingdom of Saudi Arabia.

Author Contributions

Analysis and interpretation of patient data and literature review: ASA, HAM, KAA. Involved in manuscript preparation: ASA, HAM, KAA, FFA. All authors read and approved the final manuscript.

Abbreviations

LEP: leptin
POMC: proopiomelanocortin
LEPR: leptin receptor
PC1: proconvertase 1
MC4R: melanocortin 4 receptor
PCR: polymerase chain reaction
T4: thyroxine
TSH: thyroid-stimulating hormone
FSH: follicle-stimulating hormone
LH: luteinizing hormone.

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