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. 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. 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. Of all monogenic forms of obesity, the only one causally treatable is congenital leptin deficiency caused by homozygous mutations of the leptin gene.

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. 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. It is a key regulator of energy homeostasis, by regulating energy intake and expenditure through its actions on the arcuate nucleus of the hypothalamus.

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 that of children of the same age as well as more hunger and impaired satiety seen after meals of fixed quantity and composition. 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. 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, resulting in high rates of childhood infection and therefore a high rate of childhood mortality. In those who survive, obesity continues in adult life, with hepatic steatosis and hyperinsulinemia consistent with the severity of obesity. 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. She 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. Of all monogenic genes causing obesity, LEP is the most severe and is usually lethal in early life. In this study, we have presented a case of a Saudi child with severe obesity due to a homozygous frameshift mutation leading to a premature termination of the protein LEP, which is responsible for weight control.

| VARIABLES | AGE | REFERENCE RANGE |
|-----------|-----|-----------------|
| Weight (kg) | 11 MONTHS | 2½ YEARS |
| Height (cm) | 33 |
| BMI (kg/m²) | >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 cholesterol mg/dL | 172 | 171 | <170 |
| Triglycerides mg/dL | 155 | 152 | <150 |
| LH IU/L | 0.1 | 0.5 | 0.1–3.3 |
| FSH IU/L | 15 | 20 | 10–35 |
| ALT U/L | 26 | 27 | <29 |
| T4 pmol/L | 13.3 | 15.4 | 12–22 |
| TSH uIU/ml | 2.31 | 3.01 | 0.27–4.20 |
| 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 |

| PRIMER | SEQUENCE | ANNEALING TEMPERATURE | PRODUCT SIZE (BP) |
|--------|----------|-----------------------|------------------|
| Exon 2 Forward | GTC TGG TAA TGT GGT TGG TAA T | 58 °C | 364 |
| Reverse | TCT AGG AGG CGT TCA ATA AAT G | 58 °C | 364 |
| Exon 3 Forward | CTG AGC CAA AGT GGT GAG G | 58 °C | 591 |
| Reverse | GTG TCC ATG CAA TGC TCT TC | 58 °C | 591 |
forms of obesity, the only one causally treatable is congenital leptin deficiency caused by homozygous mutations of the leptin gene.\textsuperscript{4} Mutations in \textit{LEP} genes are autosomal recessively inherited.\textsuperscript{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 \textit{LEP} gene (c.144delins TAC), which led to a frameshift and a premature termination codon (Gln49Thrfs*23). Eight mutations in the \textit{LEP} gene have been reported to be associated with congenital leptin deficiency in human beings.\textsuperscript{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 \textit{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.\textsuperscript{13} found that a total of 30 patients had been reported to carry mutations in the \textit{LEP} gene, and most of the patients described had consanguine parents. \textit{LEP} mutation associated with congenital obesity has been identified and reported in patients of Pakistani,\textsuperscript{10,17–20} Turkish,\textsuperscript{21–24} Egyptian,\textsuperscript{25} Indian,\textsuperscript{26} Turkmenistani,\textsuperscript{27} and Austrian\textsuperscript{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 \textit{LEP} gene could result in a bioinactive form of the hormone in the presence of apparently appropriate leptin levels.\textsuperscript{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 \textit{LEP} mutations has been reported as very high at 58\%, compared with 45\% for equally obese children of the same age.\textsuperscript{32} \textit{LEP} is expressed in adipose tissue, and its product leptin affects food intake and energy expenditure. Therefore, mutations in \textit{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.\textsuperscript{13} Moreover, leptin replacement therapy has been reported to reverse endocrine and metabolic alterations associated with leptin deficiency.\textsuperscript{32}

Leptin deficiency has also been associated with hypothalamic hypothyroidism characterized by a low free T4 and high serum levels of bioinactive TSH.\textsuperscript{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.144delins TAC (Gln49Thrfs*23) in exon 2 of the \textit{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
- PCT: proconvertase 1
- MC4R: melanocortin 4 receptor
- PCR: polymerase chain reaction
- T4: thyroxine
- TSH: thyroid-stimulating hormone
- FSH: follicle-stimulating hormone
- LH: luteinizing hormone.

**REFERENCES**

1. Ramachandrapa S, Farooqi IS. Genetic approaches to understanding human obesity. J Clin Invest. 2011;121(6):2080–6.
2. González-Jíménez E, Aguilar Cordero MJ, Padilla López CA, García García I. Monogenic human obesity: role of the leptin-melanocortin system in the regulation of food intake and body weight in humans. An Sci Sanit Natour. 2012;35(2):285–93.
3. Mantzoros CS, Magkos F, Brinkeretter M, et al. Leptin in human physiology and pathophysiology. Am J Physiol Endocrinol Metab. 2011;301(4):E567–84.
4. Paz-Filho G, Wong ML, Licinio J. Ten years of leptin replacement therapy. Obes Rev. 2011;12(5):e315–23.
5. Isse N, Ogawa Y, Tamura N, et al. Structural organization and chromosomal assignment of the human obese gene. J Biol Chem. 1995;270(46):27728–33.
6. Baratta M. Leptin – from a signal of adiposity to a hormonal mediator in peripheral tissues. Med Sci Monit. 2002;8(12):RA262–92.
7. Keleidis T, Keleidissi I, Chou S, Mantzoros CS. Narrative review: the role of leptin in human physiology: emerging clinical applications. Ann Intern Med. 2010;152(2):93–100.
8. Friedman JM. Leptin and the regulation of body weight. Keio J Med. 2011;60(1):1–9.
9. Velloso LA. The hypothalamic control of feeding and thermogenesis: implications on the development of obesity. Arq Bras Endocrinol Metabol. 2006;50(2):165–76.
10. Farooqi IS, Matearce G, Lord GM, et al. Beneficial effects of leptin on obesity, T cell homeostasis and receptor expression in a mouse model of human congenital leptin deficiency. J Clin Invest. 2002;110(8):1093–103.
11. Farooqi IS. The severely obese patient—a genetic work-up. Nat Clin Pract Endocrinol Metabol. 2006;2(3):172–7.
12. Dohn C, Clement K. Leptin and leptin receptor-related monogenic obesity. Biochimica. 2012;94(10):2111–5.
13. Funcke JB, von Schnurbein J, Lennerz B, et al. Monogenic forms of childhood obesity due to mutations in the leptin gene. Mol Cell Pediatr. 2014;1(1):3.
14. Ozata M, Ouzemir IC, Licinio J. Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and central system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. J Clin Endocrinol Metab. 1999;84(10):3686–95.
15. von Schnurbein J, Heni M, Moss A, et al. Rapid improvement of leptin resistance after initiation of leptin substitution in a leptin-deficient girl. Horm Res Paediatr. 2013;79(5):310–7.
16. Yang W, Kelly T, He J. Genetic epidemiology of obesity. Epidemiol Rev. 2007;29:49–61.
17. Farooqi IS, Jebb SA, Langmack G, et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med. 1999;341(12):879–84.
18. Farooqi IS. Monogenic human obesity. Front Horm Res. 2008;36:1–11.
19. Fatima W, Shahid A, Imran M, et al. Leptin deficiency and leptin gene mutations in obese children from Pakistan. Int J Pediatr Obes. 2011;6(5–6):419–27.
20. Saeed S, Butt TA, Anwer M, Arslan M, Fronguil P. High prevalence of leptin and melanocortin-4 receptor gene mutations in children with severe obesity from Pakistani consanguineous families. Mol Genet Metab. 2012;106(1):121–6.
21. Montague CT, Farooqi IS, Whitehead JP, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature. 1997;387(6636):903–8.
22. Strobel A, Isaad T, Camoin L, Ozata M, Stroossberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet. 1998;18(3):213–5.
23. Gibson WT, Farooqi IS, Moreau M, et al. Congenital leptin deficiency due to homozygosity for the Delta133G mutation: report of another case and evaluation of response to four years of leptin therapy. J Clin Endocrinol Metab. 2004;89(10):4821–6.
24. Licinio J, Caglayan S, Ozata M, et al. Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. Proc Natl Acad Sci U S A. 2004;101(13):4531–6.
25. Paz-Filho GJ, Babikian T, Asarnow R, et al. Leptin replacement improves cognitive development. Pediatr Obes. 2008;3(3):c3098.
26. Thakur S, Kumar A, Dubey S, Saxena R, Peters AN, Singhal A. A novel mutation of the leptin gene in an Indian patient. Clin Genet. 2014;84(4):391–3.
27. Chekhranova MK, Karpova SK, Iatsyshina SB, Pankov IA. A new mutation in the leptin gene in an Indian patient. Clin Genet. 2007;72(2):122–5.
28. Mazen I, El-Gammal M, Abdel-Hamid M, Amr K. A novel homozygous missense mutation of the leptin gene (N103 K) in an obese Egyptian patient. Mol Genet Metab. 2005;87(1):50–8.
29. Fischer-Posovszky P, von Schnurbein J, Moepps B, et al. A new missense mutation associated with hypogonadism and morbid obesity. Mol Genet Metab. 1997;60(1):1–9.
30. Mazen I, El-Gammal M, Abdel-Hamid M, Amr K. A novel homozygous missense mutation of the leptin gene (N103 K) in an obese Egyptian patient. Mol Genet Metab. 2005;87(1):50–8.
31. Farooqi IS, Bullmore E, Kroach J, Gillard J, O’Rahilly S, Fletcher PC. Leptin regulates striatal regions and human eating behavior. Science. 2007;317(5843):1355.
32. Paz-Filho G, Mastronardi C, Delibasi T, Wong ML, Licinio J. Congenital leptin deficiency: diagnosis and effects of leptin replacement therapy. Arq Bras Endocrinol Metabol. 2010;54(6):690–7.