Case Report

Severe early onset obesity due to a novel missense mutation in exon 3 of leptin gene in an infant from Northwest India

Short title: Leptin gene mutation in an Indian infant

Authors, their academic degrees, designations and affiliations:

1. Devi Dayal, MD, Professor, Pediatric Endocrinology Unit, Department of Pediatrics, Postgraduate Institute of Medical Education and Research, Chandigarh.
2. Keerthivasan Seetharaman, MD, Registrar, Pediatric Endocrinology Unit, Department of Pediatrics, Postgraduate Institute of Medical Education and Research, Chandigarh.
3. Inusha Panigrahi, DM, Professor, Genetic-Metabolic Unit, Department of Pediatrics, Postgraduate Institute of Medical Education and Research, Chandigarh.
4. Balasubramaniyan Muthuvel, MD, Registrar, Pediatric Endocrinology Unit, Department of Pediatrics, Postgraduate Institute of Medical Education and Research, Chandigarh.
5. Ashish Agarwal, MD, Resident doctor, Pediatric Endocrinology Unit, Department of Pediatrics, Postgraduate Institute of Medical Education and Research, Chandigarh.

Address for correspondence and reprint requests:

Dr. Devi Dayal
Professor, Pediatric Endocrinology Unit, Department of Pediatrics, Advanced Pediatrics Center, Postgraduate Institute of Medical Education and Research, Chandigarh-160012, India.

Tel: 0091-172-2755657 (O)
0091-172-2772777 (R)
Fax: 0091-172-2744401: 2745078
E-mail: drdevidayal@gmail.com

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What is already known on this topic?

Congenital leptin deficiency due to mutations of leptin (LEP) gene is a rare cause of early-onset obesity (EOO) with less than 50 cases reported so far.

What this study adds?

This article presents a new patient with severe EOO caused by a novel mutation in the LEP gene in an Indian infant.
Abstract

Monogenic obesity caused by mutations in one of the several genes involved in the control of hunger and satiety is a rare cause of early-onset obesity (EOO). The most common of the single gene alterations affect the LEP gene resulting in congenital leptin deficiency that manifests as intense hyperphagia, EOO and severe obesity associated hormonal and metabolic alterations. Only eight mutations of LEP gene associated with congenital leptin deficiency in humans have been described. In this study, we report a novel homozygous missense mutation in exon 3 of the LEP gene (chr7:127894610;c.298G>A) resulting in the amino acid substitution of Asparagine for Aspartic acid at codon 100 (p.Asp100Asn) in a 10-month-old infant who presented to us with severe hyperphagia, EOO and had low serum leptin concentrations. Additionally, a homozygous missense variation of unknown significance in exon 11 of BBS1 (Bardet-Biedl syndrome-1) gene (chr11:66291279; G>A; Depth 168x) was detected. Significant abnormalities of lipid parameters were also present in our patient. Both parents were thin built but there was a family history suggestive of EOO in a paternal uncle and a cousin. In conclusion, we report only the second patient from India with a novel mutation of the LEP gene associated with severe obesity.

Keywords: Congenital leptin deficiency, monogenic obesity, leptin gene, novel mutation, early onset obesity, India

Introduction

Severe early onset obesity (EOO) may be caused by alterations in genes that regulate appetite, body weight and energy homeostasis (1). The most common single gene alterations that cause severe EOO include mutations in the leptin (LEP), leptin receptor (LEPR), preopiomelanocortin (POMC), prohormone convertase 1 (PCSK1) or melanocortin 4 receptor (MC4R) genes which together account for 3-5% of non-syndromic cases (1). These genes are involved in the control of hunger and satiety through the leptin-melanocortin signaling pathway in the hypothalamus. Of all these genes, the most commonly affected is the LEP gene. Homozygous mutations in the LEP gene cause the recessively inherited congenital leptin deficiency which manifests as severe EOO (1). Other characteristic manifestations include impaired satiety, intense hyperphagia, a normal birth weight and rapid weight gain during early infancy (1). These children also develop several hormonal and metabolic abnormalities associated with obesity in older children and adults (2). In addition, they may have reduced T-cell number and function resulting in increased predisposition to infections and high rates of mortality during childhood (3). After the first report of a frameshift mutation in LEP gene in two severely obese cousins from a consanguineous UK family of Pakistani origin (4), several other patients with frameshift, missense or deletion mutations in LEP gene have been reported (5-17). These reports of LEP gene mutations have emanated from several countries and especially from regions with high rates of consanguinity (5-17). A vast majority (approximately 80%) of about 50 patients described in literature so far come from Central Pakistan (4-8). In this communication, we report a novel homozygous missense mutation in LEP gene associated with low serum leptin concentrations, hyperphagia and severe EOO in an infant from Northwest India.

Case report

A 10 month old girl was referred to our Endocrine Unit for evaluation of excessive and rapid weight gain. She was born at full term by normal vaginal delivery and weighed 3.0 kg at birth. She is the second child of healthy, non-obese parents with third degree consanguinity. There was no history to suggest gestational diabetes, hypertension, hypothyroidism or excess weight gain by mother during pregnancy. Parents noticed increased appetite at about 2 months of age. She started demanding feeds at half to one hourly intervals. Subsequently, there was a rapid gain in her weight to 9.5 kg at 4 months and 15 kg at 6 months
of age. There was no history of lethargy, dryness of skin, constipation, excessive hair growth, seizures, visual or sleep disturbances. There was a history of EOO in a paternal uncle and a cousin brother.

Physical examination revealed generalized body fat distribution, rounded face and deep skin folds (Fig.1A, B)). There were no stigmata of a syndrome or underlying endocrinopathy except acanthosis nigricans in axillae and neck folds (Fig.1C). The vital parameters were normal. Her weight was 19 kg (+7.38 Z-score), length 71.0 cm (-0.24 z-score) and body mass index (BMI) 37.7 kg/m² (+10.94 z-score). Anthropometric calculations were done with WHO Anthroplus software (version 1.0.4 WHO, Geneva, Switzerland). Ophthalmological evaluation did not show retinitis pigmentosa. The systemic examination was unremarkable.

Laboratory investigations revealed normal routine hematological and biochemical parameters except serum aminotransferases. The results of other laboratory evaluation are shown in Table 1. Abdominal ultrasound showed normal morphology of kidneys, liver span of 12 cm (normal 6.3-9.6 cm) and features of hepatic steatosis. Magnetic resonance imaging of brain with fine cuts through the pituitary and hypothalamus showed no abnormality. In view of intense hyperphagia followed by rapid weight gain, early age of onset, family history of EOO and low level of circulating leptin, a diagnosis of monogenic obesity due to *LEP* gene mutation was considered. Written informed consent was obtained from the parents of the patient to carry out all the laboratory studies and for publishing clinical information and photographs.

For genetic studies, genomic DNA extracted from blood was used to perform targeted gene capture using a custom capture kit on Illumina HiSeq 2000 sequencing platform (Illumina California, USA). Sequencing identified a homozygous missense mutation in exon 3 of the *LEP* gene (chr7:127894610;c.298G>A) resulting in the amino acid substitution of Asparagine for Aspartic acid at codon 100 (p.Asp100Asn). Validation of the identified mutation was done by Sanger sequencing to exclude false positivity (Fig.2). The Asp100Asn variant lies in the functional domain of the leptin protein and has not been reported in the 1000 Genomes database. It has a minor allele frequency of 0.0008% in the Exome Aggregation Consortium (ExAC) database. The *in silico* predictions of the mutation are probably damaging by Polyphen-2 (HumDiv) and damaging by SIFT (Sorting Intolerant From Tolerant), LRT (Log ratio test) and MutationTaster2. The reference codon is conserved across species.

Sequencing also revealed a homozygous missense variation in exon 11 of *BBS1* (Bardet-Biedl syndrome-1) gene (chr11:66291279;G>A; Depth 168x) resulting in amino acid substitution of isoleucine for valine at codon 346 (p.Val346Ile). This variant has a minor allele frequency of 0.16% and 0.1% in the 1000 Genomes and Exac databases respectively. The *in silico* prediction of the mutation is damaging by only MutationTaster2. The reference codon is conserved across mammals. This *BBS1* mutation is classified as a variant of uncertain significance based on the above evidence. Sanger sequencing of exon 3 of the *LEP* gene and exon 11 of *BBS1* gene of the unaffected parents identified same variations as in the index patient but in a heterozygous condition.

**Discussion**

Majority of the children with EOO have simple obesity (18). However, about 5% of all children with EOO may have monogenic obesity caused by mutations in one of the several genes involved in the regulation of appetite and body weight (1). Even rarer are the syndromic forms of EOO such as BBS, Prader-Willi syndrome and Beckwith-Wiedemann syndrome caused by genetic, epigenetic and genomic alterations (1). The most common and treatable form of monogenic obesity is due to mutations in the *LEP* gene manifesting as hyperphagia and rapid weight gain starting from early infancy (4). The clinical manifestations in the index patient were similar to the previously reported patients (4-8). Additionally, our patient exhibited severe abnormalities of lipid parameters, usually found in patients with congenital leptin deficiency during late childhood or even adulthood, at 10 months of age (8). However, other common
obesity associated complications such as abnormalities of glucose homeostasis and blood pressure were not detected in our patient (2, 8). The mild elevation of serum aminotransferases was possibly related to hepatic steatosis commonly seen in patients with obesity (19).

The mutation (chr7:127894610;c.298G>A) in our patient that lead to amino acid substitution of Asparagine for Aspartic acid at codon 100, has not been described before. However, a different missense variation (Asp100Tyr) affecting the same codon has been reported (13). Interestingly, the affected patient had high circulating levels of mutant leptin (functional studies showed that leptin was biologically inactive) (13), unlike the characteristically absent or nearly absent circulating leptin in LEP gene mutations (5-8). We presume that the low serum leptin concentrations secondary to the mutated LEP gene resulted in severe hyperphagia and severe EOO in our patient. The serum concentrations of leptin were even lower in comparison to the recent local normative data of children (mean serum levels 1.4±0.5, range 1.04–3.71 ng/mL)(20).

Leptin is an important afferent peripheral humoral signal to appetite regulating network in hypothalamus and affects food intake and energy expenditure. It is an important predictor of weight gain even during early infancy (21). Therefore, low levels of leptin or its biological inactivity resulting from mutations in LEP gene can possibly disturb the metabolic balance leading to severe obesity and related metabolic disorders. Leptin replacement normalises these hormonal and metabolic alterations suggesting that leptin deficiency or inactivity is the predominant determinant of obesity associated disorders in these patients (3, 9, 13).

The finding of BBS1 gene mutation in our patient is intriguing. BBS is a known cause of syndromic form of EOO and BBS proteins are required for leptin receptor signalling (22). However, leptin resistance rather than leptin deficiency is the characteristic finding in obese patients with BBS (23). The obesity usually manifests by 2-3 years of age unlike during early infancy in patients with LEP gene mutation (22, 23). Furthermore, our patient did not show the usual BBS stigmata such as retinitis pigmentosa, kidney dysfunction, polydactyly, behavioural problems and hypogonadism (22). Hence, the contribution of BBS1 gene mutation to obesity in our patient seems insignificant.

A significant majority of the previously reported patients belong to consanguineous families of Arain tribe located in Central Punjab, Pakistan (5-7). Incidentally, our patient hails from a geographical area in Indian Punjab close to (approximately 30 miles) the location from where most patients have been described (5-7). Although families of the Arain community have a scattered presence across Northern India including Punjab, their consanguinity rates are lower. Our patient does not belong to the Arain community although third degree consanguinity was present. The first reported patient from India also belonged to North India (16). The geographic location points to either the operation of natural selection (carrier advantage) or random genetic drift (chance founder effects) for LEP gene in this population. The limitations of our study include the lack of functional studies to understand the mechanism of disease manifestations in the patient. Also, we could not screen other affected family members for the mutation detected in our patient.

In summary, we report an infant with congenital leptin deficiency due to a novel mutation of the LEP gene manifesting as severe EOO and dyslipidemia. Ours is only the second case from India with LEP gene mutation in the published literature. In patients with EOO, identifying those with LEP gene mutations is important as recombinant human leptin therapy offers substantial clinical benefits in these patients.

Authorship Contribution

Concept: Devi Dayal

Data Collection or Processing: Keerthivasan Seetharaman, Balasubramaniyan Muthuvel, Ashish Agarwal
References


Table 1: Hormonal, metabolic and other laboratory data of the patient

<table>
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<tr>
<th>S.no</th>
<th>Variable</th>
<th>Patient’s value</th>
<th>Reference range</th>
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<tr>
<td>1.</td>
<td>Fasting blood glucose (mg/dL)</td>
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<td>70-100</td>
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<td>2.</td>
<td>Fasting C-peptide (ng/mL)</td>
<td>4.08</td>
<td>1.1-4.4</td>
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<td>3.</td>
<td>Fasting plasma Insulin (mIU/L)</td>
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<td>2.6-24.9</td>
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<td>4.</td>
<td>HbA1c (%)</td>
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<td>4.0-5.8</td>
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<td>5.</td>
<td>Plasma cortisol (nmol/L)</td>
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<td>171-536</td>
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<tr>
<td>6.</td>
<td>Plasma ACTH (pg/mL)</td>
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<td>5.0-60</td>
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<td>7.</td>
<td>Triiodothyronine (ng/mL)</td>
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<td>8.</td>
<td>Thyroxine (µg/dL)</td>
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<td>4.8-12.7</td>
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<td>9.</td>
<td>TSH (µIU/mL)</td>
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<td>25-hydroxy vitamin D (ng/mL)</td>
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<td>16.</td>
<td>Parathyroid hormone (pg/mL)</td>
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<td>17.</td>
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<td>18.</td>
<td>Low-density lipoprotein cholesterol (mg/dL)</td>
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<td>19.</td>
<td>High-density lipoprotein cholesterol (mg/dL)</td>
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<td>Serum leptin (ng/mL)</td>
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<td>21.</td>
<td>Serum adiponectin (mg/mL)</td>
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<td>Aspartate Aminotransferase (U/L)</td>
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<td>3-30</td>
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<tr>
<td>23.</td>
<td>Alanine aminotransferase (U/L)</td>
<td>89</td>
<td>7-45</td>
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</table>

**Legends for figures**

**Fig. 1:** Clinical photographs of the patient showing generalized body fat distribution (A), deep skin folds (B) and acanthosis nigricans (C).

**Fig. 2:** Sequence chromatogram showing homozygous missense mutation in exon 3 of the *LEP* gene (chr7:127894610:c.298G>A) resulting in the amino acid substitution of Asparagine for Aspartic acid at codon 100 (p.Asp100Asn).