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Clinical and molecular characterisation of children with monogenic obesity: a case series

Charakterystyka kliniczna i molekularna dzieci z otyłością monogenową: seria przypadków

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Abstract

Objective: To study the clinical profile and molecular diagnosis of children with severe early-onset non-syndromic monogenic obesity.

Methods: The clinical and molecular data (performed using whole exome sequencing) of 7 children with early-onset (< 5 years) non-syndromic monogenic obesity were extracted from the Obesity Clinic files and analysed retrospectively.

Results: The median (IQR) age at presentation was 18 (10.5–27) months. Of the 7 patients, 5 were boys, 3 had a history of parental consanguinity, and 4 had a family history of severe early-onset obesity. All patients exhibited hyperphagia and showed signs of insulin resistance. Dyslipidaemia and fatty liver were observed in 4. The variants identified in 6 patients included 2 in leptin receptor, and one each in melanocortin 4 receptor, pro-opiomelanocortin, leptin, and neurotrophic tyrosine kinase receptor type 2 genes. Notably, 4 of these variants were novel.

Conclusion: This case series provides valuable insights into the spectrum of genetic mutations associated with non-syndromic monogenic obesity in North Indian children. The findings underscore the significance of next-generation sequencing in identifying the aetiology of severe early-onset obesity.

Key words: early-onset obesity, monogenic obesity, leptin-melanocortin pathway, novel mutations.

Introduction

Childhood obesity has emerged as a striking global health issue, with a steady increase in its prevalence in children under the age of 5 years, from 4.8% in 1990 to 5.9% in 2018 [1]. Although most cases of childhood obesity can be attributed to exogenous causes, approximately 3–10% of those with severe early-onset obesity (EOO) are due to genetic disorders [2, 3]. The exact prevalence of monogenic obesity remains uncertain because this aspect of childhood obesity remains relatively understudied, and missed diagnoses are common [4].

The genetic causes of EOO are classified as syndromic and non-syndromic. Examples of syndromic EOO include Prader-Willi, Bardet-Biedl, Cohen, and Alström syndromes, whereas non-syndromic EOO may be monogenic, polygenic, or chromosomal. Non-syndromic monogenic obesity is caused by mutations in genes involved in appetite regulation, energy metabolism, and satiety control, particularly within the leptin-melanocortin pathway [5]. Among the various genes responsible for non-syndromic monogenic obesity, the melanocortin-4 receptor (*MC4R*) gene is the most common, accounting for approximately 4–6% of cases [5, 6]. Mutations in the leptin receptor gene (*LEPR*) contribute to another 3%, while mutations in genes such as pro-opiomelanocortin (*POMC*), leptin (*LEP*), proprotein convertase subtilisin/kexin type 1 (*PCSK1*), neurotrophic tyrosine kinase receptor type 2 (*NTRK2*), brain-derived neurotrophic factor (*BDNF*), and single-minded family bHLH transcription factor 1 (*SIM1*) are rare causes of monogenic obesity [3–6]. It is vital to make an exact diagnosis of genetic obesity to allow patient-tailored treatment as new drugs that target appetite regulation and energy expenditure are becoming available. Additionally, identifying the genetic causes during childhood helps in disease prognostication and genetic counselling for the family. However, the data on genetic obesity are scarce from low-income countries like India. Herein, we describe the clinical profile and specific genetic mutations ob-

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served in 7 children diagnosed with monogenic obesity at our institution.

Methods

Our study involved a retrospective analysis of the medical records of all children presenting with EOO at a tertiary care centre in Chandigarh, India, from January 2017 to December 2022. The inclusion criteria were children with onset of obesity below 5 years of age. Obesity was defined as BMI or weight-for-height or length exceeding 3 standard deviation score (SDS) by the World Health Organisation (WHO) standards for children below 5 years of age. Patients with syndromic forms of obesity were excluded based on clinical evaluation. The collected data included demographic characteristics, family history, anthropometric measurements, lipid profile, HbA1c, abdominal ultrasonography findings to assess for fatty liver, and polysomnography results for children with symptoms suggestive of obstructive sleep apnoea (OSA). Genetic mutation analysis was performed after obtaining informed consent from the parents. Consent was taken from 6 families comprising 7 patients, which was performed by a private laboratory. Because point mutations have been found to be the predominant form of variants in monogenic cases of obesity, and with advanced next generation sequencing (NGS) algorithms now being capable of detecting copy number variants as well, whole exome sequencing (WES) was used as the genetic test of choice in present study.

Bioethical standards

The study was approved by the Institute's Ethics Committee (INT/IEC/2024/000791). Consent was taken from 6 families comprising 7 patients.

Results

The median (IQR) age at presentation was 18 (10.5–27) months. Out of the 7 cases, 4 had a positive family history of EOO, and Patients 2 and 7 were siblings. Patients 1, 5, and 6 were born to third-degree consanguineous parents. Hyperphagia was a predominant symptom in all 7 cases. Additionally, all children exhibited features of insulin resistance. Dyslipidaemia and fatty liver were observed in 57.1% of the patients. Patient 4 presented with ambiguous genitalia in addition to obesity (Table I).

NGS revealed specific genetic variants: Patient 1 exhibited a homozygous pathogenic mutation in the *MC4R* gene, while Patients 2 and 5 had homozygous mutations in the *LEPR* gene. In Case 3, a heterozygous exon duplication in the *NTRK2* gene was detected, while a heterozygous deletion in the *POMC* gene was identified in Patient 4. Patient 6 (previously reported) had a homozygous variant in the *LEP* gene (Table II) [7].

We identified 4 novel variants. First, the homozygous nonsense variation in exon 1 of the *MC4R* gene (chr18:g.60372303C>T) results in the replacement of the amino acid tryptophan by a premature stop codon (Ter), which leads to the premature truncation of the protein at codon 16. Second, homozygous 5' splice variation in Intron 3 of the *LEPR* gene (chr1:g.65565606G>A) affects the invariant GT donor splice site of exon 3 (c.40+1G>A). Third, the deletion of nucleotide c.726 in the *POMC* gene leads to a frameshift mutation that affects amino acid serine at position 243 and results in premature termination of the protein at position 9; 'in silico' tools confirmed that this novel variant is disease causing. Fourth, the mutation in exon 3 of the *LEP* gene results in an amino acid substitution, with aspartic acid being replaced by asparagine at codon 100 (p.Asp100Asn).

Discussion

The assessment of a child with EOO entails a detailed history and careful physical examination. Although most of these children have exogenous or simple obesity, it is essential to identify the 'red flags', i.e. clinical pointers that may suggest an underlying monogenic aetiology (Table III). History should include birth weight, age of onset of obesity and the tempo of weight gain, dietary intake, feeding pattern, patterns and duration of physical activity, medication intake, developmental history, and family and psychosocial history. Because hyperphagia is a distinctive symptom in most genetic forms of obesity, its objective assessment must be carefully sought. Caregivers should be questioned specifically regarding the food-seeking behaviour of the child, including obtaining food by manipulation or stealing, seeking food from the trash, eating food that is normally considered as lacking taste, ability to be distracted from food-related thoughts, lack of satiety after a full meal, and distress on denial of food. In addition, a history suggestive of obesity-related complications, like obstructive sleep apnoea (headache, snoring, disturbed sleep at night, excessive daytime sleepiness), pseudotumor cerebri (headache, vision problems), slipped capital femoral epiphyses (hip pain, limping gait), etc., should be taken. Physical examination should include an assessment of general appearance, anthropometry (weight, length/height, BMI, midparental height, waist circumference), blood pressure, presence or absence of dysmorphism, skin or hair abnormality, microcephaly, polydactyly and hypogonadism.

The present study highlights the genetic heterogeneity of non-syndromic monogenic obesity, with the identification of the variants involving the *MC4R, LEPR, LEP, POMC,* and *NTRK2* genes. MC4R deficiency is the most common cause of monogenic obesity [3–6]. Children with MC4R deficiency present with severe EOO, hyperphagia, hyperinsulinaemia, and tall stature. Our patient with the *MC4R* gene variant also presented with progressive weight gain and hyperphagia since 2 months of age and had a +11 BMI SDS by 1.5 years of age. He also showed features of insulin resistance in the form of severe acanthosis nigricans. However, he did not have tall stature.

Case 2 displayed a novel splice site variant *LEPR*: c.40+1G>A, which is predicted as pathogenic on some in-silico tools and VUS on other tools, as well as a phylo-P100 score of 4.338; whereas case 5 had a missense variant in the *LEPR* gene. Both had hyperphagia and rapid weight gain noted

Table I. Demographic, clinical, and anthropometric parameters of patients

Case no.	City/ State	Age at presentation (months)	Gender	Age at onset of obesity (months)	Consanguinity/ Family history	Hyperphagia	Anthropometry at presentation			Complications
							Weight (kg), WFA (SDS)	Height (cm), HFA (SDS)	BMI kg/m^2, BMI SDS	
1.	Rajouri, J&K	18	Male	2	Yes/Yes	Yes	26.2 9.80z	86.5 1.51z	35.92 11.12z	Dyslipidaemia/ Grade II fatty liver/ OSA/AN
2.*	Alampur, Bihar	45	Male	3	No/Yes	Yes	37.6 12.08z	108 1.38z	35.69 12.72z	Dyslipidaemia/ Grade II fatty liver/ Mild OSA/AN
3.	Kakru, Haryana	11	Male	1	No/No	Yes	13.95 3.7z	73.7 –0.36z	25.7 5.14z	AN
4.	Ramnagar, Haryana	6	Male	1	No/No	Yes	16.2 4.05z	82 0.72z	24.09 4.72z	AN
5.	Sirsa, Haryana	28	Male	1	Yes/No	Yes	25.1 6.14z	90.4 –0.16z	30.71 9.1z	AN
6.	Punjab	10	Female	2	Yes/Yes	Yes	19 7.38z	71 –0.24z	37.7 10.94z	Dyslipidaemia/ Fatty liver/AN
7.*	Alampur, Bihar	26	Female	3	No/Yes	Yes	23.6 5.57z	86.6 –0.31z	31.47 8.81z	Dyslipidaemia/ Grade II fatty liver/ Mild OSA/AN

*Cases 2 and 7 are siblings.
WFA – weight for age; HFA – height for age; SDS – standard deviation scores; OSA – obstructive sleep apnoea; AN – acanthosis nigrican

Table II. Molecular analysis of the cases

Gene	Location	Variant	Zygosity	Inheritance	Classification	ACMG Criteria	Type of mutation	Comment on mutation
MC4R	Exon 1	c.47G>A	Homozygous	AR	Pathogenic	PVS1 PM2	Nonsense	Novel Only 9 nonsense variants have been reported in Clinvar till now
LEPR (+)	Intron 3	c.40+1G>A (5' splice site)	Homozygous	AR	Likely Pathogenic	PVS1 PM2	Splice Site	Novel Only 3 splice site variants have been reported in Clinvar till now
NTRK2	Exon 13-19	Chr9:g. (84752086_84861039)_ (85025751_?)dup	Heterozygous	AR	Likely Pathogenic	2K, 4E, 5G criteria met 0.5 points	CNV of (Duplication) 165 Kb	Needs CMA for confirmation and better estimation of size and breakpoints
POMC (+)	–	C.726del p.Ser243ProfsTer9	Heterozygous	AR	Uncertain significance	PVS1 PM2	Deletion	Novel Carriers are also known to be symptomatic
LEPR (+)	Exon 11	c.1418G>C (p.Cys473Ser)	Homozygous	AR	Uncertain significance	PM2 PP3 BP1	Missense	VUS on ClinVar – Single Submitter, phenotype not described
LEP	Exon 3	chr7:127894610; c.298G>A	Homozygous	AR	Likely Pathogenic	PP3 PM5 PM2 PP2	Missense	Reported first by our centre in 2018

MC4R – melanocortin-4 receptor; LEPR – leptin receptor; NTRK2 – neurotrophic tyrosine kinase receptor type 2; POMC – pro-opiomelanocortin; LEP – leptin; AR – autosomal recessive; ACMG – American College of Medical Genetics and Genomics; CNV – copy number variation; CMA – chromosomal microarray analysis; VUS – variant of uncertain significance

Table III. Diagnostic clues or 'red flags' for monogenic non-syndromic obesity

Red flags in history or examination	Probable aetiology
Hyperphagia, severe obesity with a BMI SDS > 3.5, rapid weight gain in the first 2 years of life	Common feature of all monogenic obesity disorders
Frequent infections, hypogonadism, neurological and endocrine dysfunction	LEP/LEPR defects
Pale skin, red hair, adrenal insufficiency, cholestatic jaundice	POMC defect
Tall stature, increased lean mass, hyperinsulinaemia	MC4R defect
Small bowel enteropathy, postprandial hypoglycaemia	PCSK1 defect
Developmental delay, autism-like features	SIM1 or NTRK2 defect
Memory impairment, nociception abnormalities, hyperactivity	BDNF defect

BDNF – brain-derived neurotrophic factor; LEP – leptin; LEPR – leptin receptor; MC4R – melanocortin-4 receptor; NTRK2 – neurotrophic tyrosine kinase receptor type 2; PCSK1 – proprotein convertase subtilisin-kexin 1; POMC – pro-opiomelanocortin; SIM1 – single-minded homologue 1

since early infancy. Patient 7, who was the sibling of case 2, also had a similar phenotype but did not undergo genetic testing. Family history was notable for morbid EOO in 2 paternal aunts. Patients 2 and 6 also had dyslipidaemia, fatty liver, and mild OSA. Leptin receptor deficiency is known to be associated with severe EOO, hyperphagia, hypogonadotropic hypogonadism, and endocrinological and immunological dysfunction [8]. However, no other abnormalities were detected in our cases, but this may warrant further evaluation on follow-up. The *LEPR* gene mutations are typically inherited in an autosomal recessive manner, and most reported cases in the literature have a history of parental consanguinity [9]. A high prevalence of founder mutations in the *LEP* and *LEPR* genes has been identified in consanguineous families belonging to the Arain tribe in Central Punjab, Pakistan [10]. Interestingly, consanguinity was not a predominant feature in our cases (present in only one family), and neither of our probands with *LEPR* mutations had the above-mentioned ancestry.

Genetic analysis of case 3 revealed a heterozygous duplication (copy number gain) of size (~164.72 Kb), encompassing exon 13–19 of the *NTRK2* gene. Such exon duplications in the *NTRK2* have been reported in patients with obesity, leading us to classify the heterozygous contiguous duplication variation as likely pathogenic in our case [11]. The *NTRK2* gene encodes the neurotrophin receptor TrkB, which is the cognate receptor for *BDNF* and plays a vital role in neurogenesis and maintenance of neuronal plasticity in the hypothalamus. The *NTRK2* gene mutations have been implicated in the pathogenesis of EOO, hyperphagia, and developmental delay [3, 12]. Our case did not have a developmental delay at presentation or during the last follow-up at 2 years of age; however, a longer follow-up duration would be crucial.

Patient 4 presented at 6 months of age with small penile size, under-developed scrotum, and glandular hypospadias, and he developed obesity during follow-up at 12 months of age. Genetic testing revealed a novel heterozygous mutation in the *POMC* gene, a variant of unknown significance (VUS). POMC deficiency is a rare autosomal recessive condition characterised by EOO, adrenal insufficiency, and red hair. Heterozygous mutations in *POMC* have also been associated with susceptibility to EOO [13]. In our case, the latter phenotype was observed along with hypogonadism. POMC defects can have a heterogenous phenotypic spectrum comprising varied endocrine manifestations such as hypothyroidism, type 1 diabetes, growth hormone deficiency, and hypogonadism in both sexes [14].

Patient 6 presented at the age of 10 months, exhibiting marked hyperphagia and EOO. She was born to third-degree consanguineous parentage and had a noteworthy family history marked by EOO affecting her paternal uncle and a male cousin. She had a constellation of metabolic derangements, including dyslipidaemia, hepatic steatosis, and insulin resistance. Low circulating leptin levels prompted a suspicion of congenital leptin deficiency. Subsequent molecular analysis unveiled a homozygous missense mutation located within exon 3 of the *LEP* gene (chr7:127894610;c.298G>A) [7]. Interestingly, this patient belonged to a location in the Indian state of Punjab, which is approximately only 30 miles from central Punjab, Pakistan, where leptin deficiency is quite prevalent [10].

Because children with monogenic obesity lack the physiological hunger-satiety feedback, effective weight management with nutritional and lifestyle management alone becomes challenging. With the advent of precision medicine, genetic testing by NGS may play a pivotal role in identifying specific gene mutations that may be amenable to pharmacotherapy. For instance, the use of recombinant leptin analogue (metreleptin) in children with congenital leptin deficiency or dysfunction has

been shown to aid not only in the reduction of energy intake and fat mass but also in the improvement of metabolic and endocrine abnormalities [15]. Similarly, setmelanotide (MC4R agonist) has been approved for chronic weight management in children 6 years and older with obesity secondary to LEPR, POMC, or PCSK1 defects [16]. Although these drugs offer promise in the management of monogenic obesity, availability and cost limit their use in low-income settings.

The limitation of this study was the relatively small number of cases, which may restrict the exploration of the prevalence and phenotypic spectrum of monogenic obesity. In addition, we could not perform functional studies in the variants.

Conclusions

In conclusion, this study adds to the spectrum of genetic variants associated with non-syndromic monogenic obesity specific to the North Indian population. Establishing the aetiology of severe early onset non-syndromic obesity by genetic testing aids in better counselling of the caregivers and in avoiding the social stigma associated with obesity.

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