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# A novel *MC4R* mutation associated with childhood-onset obesity: A case report

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The melanocortin-4-receptor gene (*MC4R*) is a key regulator of energy homeostasis, food intake and body weight. *MC4R* gene mutations are associated with early-onset severe obesity. Most patients are heterozygotes, with some reports of homozygotes and compound heterozygotes. The authors report a case involving an eight-year-old girl with progressive weight gain from infancy, body mass index 44 kg/m$^2$ (>97th percentile), hyperphagia, hyperinsulinemia and increased linear growth. There was no phenotype of morbid obesity in the parents or sibling. Coding regions and intron-exon boundaries of the genes encoding leptin, leptin receptor, pro-opiomelanocortin and *MC4R* were analyzed. Two heterozygous coding mutations in the *MCR4* gene (S94N and C293R) were detected, of which the second has not been previously reported. The mutations were on opposite chromosomes, confirming compound heterozygosity. The molecular findings and clinical features associated with this novel *MC4R* mutation are described. The authors emphasize that rare mutations can be found in some patients with severe childhood-onset obesity.

**Key Words:** MC4R; *Melanocortin; Obesity*

## Une nouvelle mutation *MC4R* associée à l'obésité se manifestant pendant l'enfance : un rapport de cas

Le gène récepteur de la mélanocortine 4 (*MC4R*) joue un rôle clé dans le maintien de l'homéostasie énergétique, de la prise alimentaire et du poids corporel. Les mutations du gène *MC4R* s'associent à une grave obésité à apparition précoce. La plupart des patients sont hétérozygotes, mais il y a certaines descriptions de patients homozygotes et d'hétérozygotes composés. Les auteurs présentent le cas d'une fillette de huit ans ayant une prise de poids progressive depuis la première enfance, un indice de masse corporel de 44 kg/m$^2$ (>97$^e$ percentile), une hyperphagie, une hyperinsulinémie et une augmentation de la croissance linéaire. Les parents et la fratrie ne possédaient pas de phénotype d'obésité morbide. Les auteurs ont analysé les régions codantes et les limites intron-exon des gènes codant la leptine, son récepteur, la pro-opiomélanocortine et le *MC4R*. Ils ont décelé deux mutations en région codante du gène *MCR4* (S94N et C293R), la deuxième n'ayant jamais été déclarée auparavant. Les mutations se situaient sur des chromosomes opposés, ce qui confirme leur hétérozygotie composée. Les auteurs décrivent les observations moléculaires et les caractéristiques cliniques associées à cette nouvelle mutation *MC4R*. Ils soulignent que certains patients ayant une importante obésité qui se manifeste pendant l'enfance peuvent présenter des mutations rares.

The melanocortin-4 receptor gene (*MC4R*) is an important regulator of energy homeostasis, food intake and body weight in the hypothalamus. *MC4R* mutations are the most common form of monogenic obesity and have been implicated in 1% to 6% of early-onset severe obesity. We report a novel *MC4R* mutation in an eight-year-old girl who presented with severe obesity.

## CASE PRESENTATION

An eight-year-old girl was referred to the paediatric endocrinology service with severe obesity. She was born at term, with a birth weight of 3.65 kg. There were no complications in the perinatal or neonatal periods. Rapid progressive weight gain to >97th percentile was reported from four months of age. She experienced continuous weight gain over the years, which was even more noticeable after seven years of age.

Hyperphagia was described from the first year of life. The child was constantly food-seeking, which became more evident after her first year. She requested to eat every 30 min. Dietary assessment at eight years of age suggested that the child was eating two to three times the recommended adult size portions based on Canada's Food Guide. Preference was given for milk/grains, with poor intake of vegetables/fruits. Despite no accurate quantified measurement of physical activity, the child was not following the minimum recommendations stated by Canadian physical activity guidelines for her age group (a minimum of 60 min of moderate-to-vigorous physical activity daily). Pubertal changes were noted three months before her first visit, with breast and pubic hair development. Adenoidectomy was performed at four years of age and tonsillectomy at eight years of age due to obstructive sleep apnea.

The patient's parents were first-degree cousins. There was no phenotype of morbid obesity in her parents, nor in her 18-year-old sibling. Her maternal grandmother had type 2 diabetes mellitus and died at 39 years of age from a myocardial infarction. A maternal uncle underwent angioplasty at 30 years of age.

Table 1 summarizes the initial clinical/biochemical investigations. Body mass index (BMI) was markedly elevated (44 kg/m$^2$ [Z-score = 3.0; >99.9th percentile]). She presented with acanthosis nigricans on her neck and in her axillary regions. She had increased hair distribution on the lower abdomen and back. Her pubertal assessment was Tanner stage II for breast and pubic hair development. The progression of anthropometric data is presented in Figure 1.

The child was enrolled in a multidisciplinary program for childhood obesity addressing dietary intake and exercise training. She was started on metformin 1000 mg twice per day.

After obtaining informed consent from the child's parents, DNA was isolated from whole blood and was subjected to Sanger sequencing to screen for monogenic forms of obesity, including coding regions, intron-exon boundaries and 5′- and 3′-untranslated regions of the genes encoding leptin, leptin receptor, pro-opiomelanocortin and the melanocortin-4-receptor (*MC4R*). Primer sequences used to perform polymerase chain reaction amplifications and sequencing are available from the authors on request. Sequencing showed that the child had two heterozygous

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**TABLE 1**
**Clinical features and investigations for an eight-year-old girl with *MC4R* mutation**

| Clinical features and initial investigations | |
|---|---|
| Height, cm | 149.2 |
| Height-to-age Z-score, percentile | +3.0 (>>99.9th percentile) |
| Weight, kg | 98.0 |
| Weight-to-age Z-score, percentile | +4.0 (>>99.9th percentile) |
| BMI, kg/m$^2$ | 44 |
| BMI-to-age Z-score, percentile | +3.0 (>>99.9th percentile) |
| SBP, mmHg | 112 (Z-score 0.7; 75th percentile) |
| DBP, mmHg | 70 (Z-score 0.7; 75th percentile) |
| 2 h oral glucose tolerance test | |
| Fasting glucose, mmol/L | 4.9 |
| 2 h glucose, mmol/L | 6.1 |
| Fasting insulin, pmol/L | 414 |
| HOMA index | 5.04 |
| Karyotype | 46XX (negative fluorescent in situ hybridization for Prader-Willi syndrome) |
| Bone age | 11 years |

*Thyroid function, adrenocorticotropic hormone and morning cortisol levels were normal (data not shown) BMI Body mass index; DBP Diastolic blood pressure; HOMA Homeostasis model assessment (index for assessment of insulin resistance; normal values for female adolescents are reported to be <3.0); SBP Systolic blood pressure.*

mutations in the *MC4R* gene, which would predict single amino acid (missense) changes in the primary amino acid sequences, namely p.S94N and p.C293R missense mutations.

## DISCUSSION

Diet and exercise changes have globally contributed to the increased prevalence of childhood obesity. Nevertheless, there is evidence of a substantial hereditary contribution to body weight based on family studies and studies of twins and adopted children (1). The genetic component includes polygenic susceptibility and inheritance of rare, large-effect gene mutations that lead to monogenic obesity syndromes.

Among the single-gene causes of severe childhood obesity, genetic mutations in the leptin-melanocortin system have been shown to cause severe obesity (2). *MC4R* mutations have been identified as the most common monogenic form of obesity and have been implicated in 1% to 6% of early-onset childhood obesity (3).

We report a case involving a child with an *MC4R* mutation. The *MC4R* gene is located on chromosome 18q21.3. It encodes a 332 amino acid protein that is a key regulator of energy homeostasis, food intake and body weight regulation at the level of the hypothalamus (3). Mutations that decrease *MC4R* activity within the melanocortin pathway generate orexigenic signals (4). *MC4R* plays an integral role within the leptin-pro-opiomelanocortin pathway regulating food intake and energy expenditure (Figure 2A) (5).

*MC4R* gene mutations are typically inherited in a dominant pattern, of which the majority present as heterozygous mutations. Both homozygous and heterozygous mutations have been identified (6,7). *MC4R* mutations generally result in decreased or absent *MC4R* activity. More than 150 missense mutations have been reported (3).

In the present case, the p.C293R mutation has not been previously reported, while the p.S94N mutation has been described associated with the obese phenotype (3). The variant p.S94N has been associated with loss of function of *MC4R* (8).

The p.C293R missense mutation was present in the proband's mother, who was phenotypically unaffected, suggesting that a single heterozygous p.C293R variant does not confer a phenotype of obesity. The penetrance of obesity in carriers of functionally significant *MC4R* variants is variable; homozygous individuals present with more severe phenotypes than heterozygous (8). The

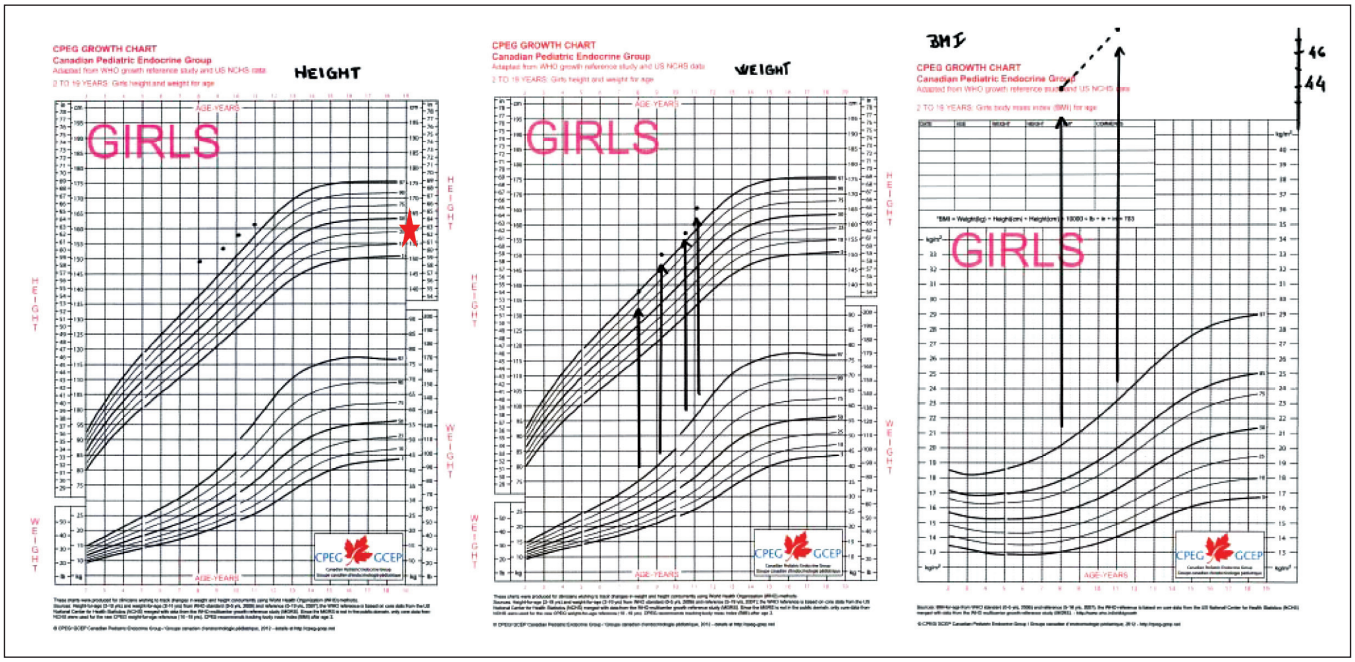


**Figure 1)** *Height, weight and body mass index (BMI). The star in the left panel represents mid-parental height. Arrows indicate the distance of measures in relation to the 97th percentiles for weight and BMI. Anthropometric data were plotted on Canadian Pediatric Endocrine Group growth charts, adapted from the WHO growth reference study and United States National Center for Health Statistics data and supported by the Canadian Pediatric Endocrine Group*

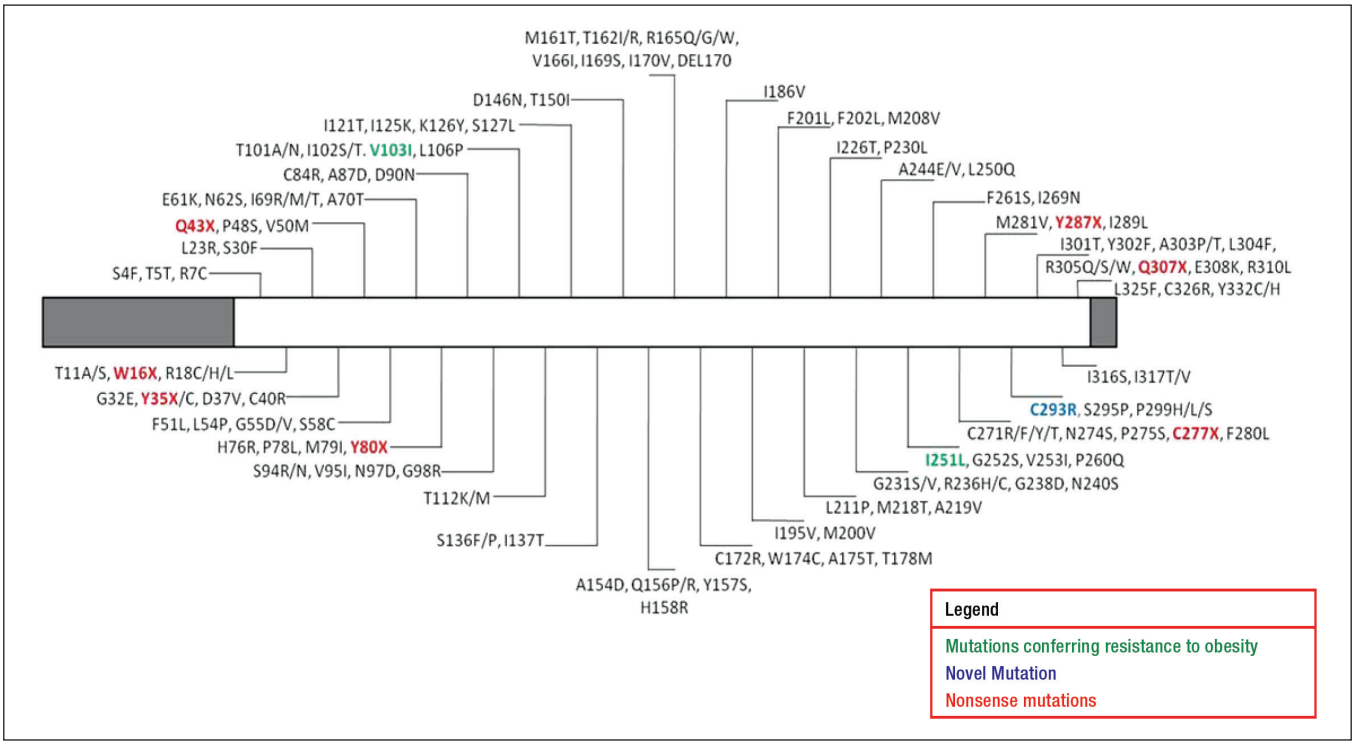


**Figure 2)** *Mutation map with the novel melanocortin-4 receptor mutation identified in blue*

proband's sister did not have a *MC4R* gene mutation. We were unable to test the proband's father.

Our findings suggest that the proband's *MC4R* mutation of p.S94N was either inherited from the father or, alternatively, the mutation arose spontaneously. The father was reported to be a nonobese adult. Although complete clinical and genetic data were not available, it would appear that simple heterozygosity for each mutation is not associated with an obvious phenotype and that a compound heterozygosity for both mutations may be required for disease expression.

*MC4R* gene mutations have been associated with childhood-onset obesity, hyperphagia and hyperinsulinism (9). In *MC4R* knockout mice, increased plasma insulin levels and impaired insulin tolerance are noted. Our patient presented with clinical and biochemical evidence of hyperinsulinism. *MC4R* knockout mice develop impaired insulin tolerance before the development of hyperphagia and obesity (10). In addition, affected individuals typically present with rapid weight gain in infancy, developing severe obesity early in childhood (BMI >>97th percentile for age). Hyperphagia is characterized by frequent food-seeking behaviour and a large intake of food during meals with poor satiety, and is observed in early infancy. Carriers develop hyperinsulinemia and increased lean mass (9).

Relevant clinical features were highlighted in the present case. The patient exhibited hyperphagia, noticed from six months of age, along with a rapid increase in weight gain and accelerated linear growth in childhood. In MC4R deficiency, increased linear growth leads to increased adult final height. In combination with hyperinsulinism, incomplete suppression of growth hormone secretion may contribute to the accelerated growth phenotype characteristic of MC4R deficiency (11).

In terms of reproductive function, female *MC4R* knockout mice exhibit advanced reproductive age; in contrast, males exhibit erectile dysfunction (12), which appears to be modifiable by exercise (13). Our patient showed early spontaneous onset of puberty.

Children with reduced MC4R activity have more difficulty with the maintenance of weight loss (14). Hyperphagia manifests early in infancy and increases with age (9). In the present case, hyperphagia was a significant feature, stressful to both the child and family members. Despite several attempts to engage this patient and family in programs aiming for lifestyle changes and promoting physical activity, the patient's BMI was 56.6 kg/m$^2$ after four years of follow-up. She developed further obesity-related complications including lymphedema, impaired glucose tolerance and moderate obstructive sleep apnea.

*MC4R* mutation is the most common form of monogenic obesity. The present case report highlights the importance of early suspicion of monogenic causes for obesity in the general paediatric community. Despite no specific therapy, early recognition of the molecular subtype leads to earlier diagnosis and to a more individualized, effective treatment involving a multidisciplinary approach. Practical points on diagnostic considerations for general paediatricians are listed below.

**When should monogenic obesity be suspected?**

- Rapid weight gain from early infancy (the most important feature);
- Development of severe obesity (BMI >>97th percentile) at early ages, usually <3 years of age;
- Persistent food-seeking behaviour, mostly reported from six months of age;
- Acanthosis nigricans or signs of hyperandrogenism (in girls) in a young child;
- Parental consanguinity (may or may not be present);
- Parental/siblings anthropometric data: suspect if relatives present normal anthropometric data. However, environmental factors contribute to obesity among genetically predisposed individuals and obesity may be a common feature in relatives;

- Tall stature/increased growth velocity (*MC4R* monogenic diabetes).

**Important considerations in assessing an overweight/obese child:**

- Paediatric patients should have height, weight and BMI plotted in appropriate charts and measured at least once per year (more often if <1 year of age);
- Search for stigmata of syndromes associated with obesity (developmental delay, dysmorphism, hypogonadism), and signs of hyperinsulinism (acanthosis nigricans) and hyperandrogenism;
- Perform basic laboratory evaluation to rule out obesity-related metabolic abnormalities: glucose intolerance, dyslipidemia and altered liver function;
- Consider a referral to a specialist in the presence of suspected syndromes associated with obesity or comorbidities associated with simple obesity;
- Primary care providers play an essential role in motivating, educating and supporting behavioural modification of obese children.

## CONCLUSION

The patient in the present case exhibited two heterozygous coding mutations in the *MC4R* gene (C293R and S94N). The first mutation has not been previously described. Paediatric endocrinology assessment for monogenic forms of obesity should be considered in the presence of rapid weight gain from infancy, early-onset severe obesity, hyperphagia and clinical evidence of hyperinsulinism.

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