Melanocortin 4 Receptor (*MC4R*) Gene Associated Severe Obesity in an Indian Child: Report of Novel Variants

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Abstract

Monogenic obesity syndromes are a group of Mendelian diseases that cause childhood obesity and are linked to genes in the leptin- melanocortin pathway. Families with biallelic *MC4R* gene mutations are rare. We describe a seventeen-year-old girl with severe obesity, diabetes, hyperinsulinemia, hypercholesterolemia, primary amenorrhea, polycystic ovary and acanthosis nigricans. Clinical exome analysis identified compound heterozygosity for variants p.(Cys84Tyr) and p.(Leu300Pro) in the *MC4R* gene encoding the melanocortin 4 receptor protein in the patient. Father was heterozygous for the p.(Leu300Pro) variant and mother was heterozygous for the p.(Cys84Tyr) variant. The patient's obesity did not respond to dietary and exercise management although the glucose control and lipid profile improved. The patient's parents were also obese but less severely affected (body mass index of father 39.2 and of mother 38.2).

Case report

A seventeen-year-old girl, born to non-consanguineous parents, presented with childhood onset hyperphagia and obesity. She was born at term by lower segment Caesarean section and her birth weight was 3.785 kilograms. There was history of gestational diabetes in the mother in the antenatal period. She had hypoglycemia and hyperbilirubinemia (5th day total bilirubin was 24.6 mg/dl) and was treated with phototherapy; at discharge her weight was 4.3 kgs. Her weight progressively increased and at 38 months, her weight was 27 kgs for height of 94 cm when she was noticed to have acanthosis nigricans, moon face and buffalo hump. Fasting cortisol and evening cortisol were normal. Bowing of legs was noticed after she started walking at 15 months of age when she was treated for rickets. Later, at 44 months of age, she was diagnosed to have Blount disease and corrective osteotomy was done at 4 years of age. Fasting lipid profile at 4 years showed serum cholesterol of 172 mg%, serum triglycerides of 212 mg%, HDL cholesterol of 37 mg %, LDL cholesterol 93 mg% and VLDL cholesterol of 42 mg%. Fasting sugar and postprandial sugar were normal. At 12 years, she weighed 111 kilos, height was 151 cm, random blood sugar was 197 mg%, serum insulin level was 1000 IU/ml (normal fasting 3 – 35 IU/ml, post glucose 12 – 82 IU/ml), SGPT was 60 IU/l (normal up to 40 IU/l), and total cholesterol/HDL cholesterol ratio was 6.7. Ultrasonogram of the abdomen revealed normal liver, spleen, kidneys, adrenal glands and uterus, but a single 21 mm large calculus was noted in the gall bladder. The left ovary showed a bulky, polycystic appearance with multiple peripheral tiny follicles and increased stromal echotexture. Serum FSH was 1.95 mIU/ml, LH was 1.46 mIU/ml, and prolactin was 9.18 ng/ml. In the last visit at 17 years of age, she weighed 137 kgs and her height was 155 cm (Figure 1a). Figure 1b shows the fat distribution in the dual-energy X-ray absorptiometry (DEXA) scan. Her cognitive functions were normal. She did not lose weight despite several attempts at low calorie diets, exercise and swimming sports regimens. Hyperphagia was noticed in early life and this remained difficult to control. The patient was advised gastric bypass surgery (Roux-en-Y) for better long-term management of obesity.

Her father was 175 cm tall, with a weight of 120 kgs and BMI of 39.2. Her mother was 160 cm tall, with weight of 72 kgs and BMI of 28.12. Her brother was 170 cm tall with a weight of 95 kgs, BMI 32.87 and was receiving treatment for hypothyroidism diagnosed in childhood.

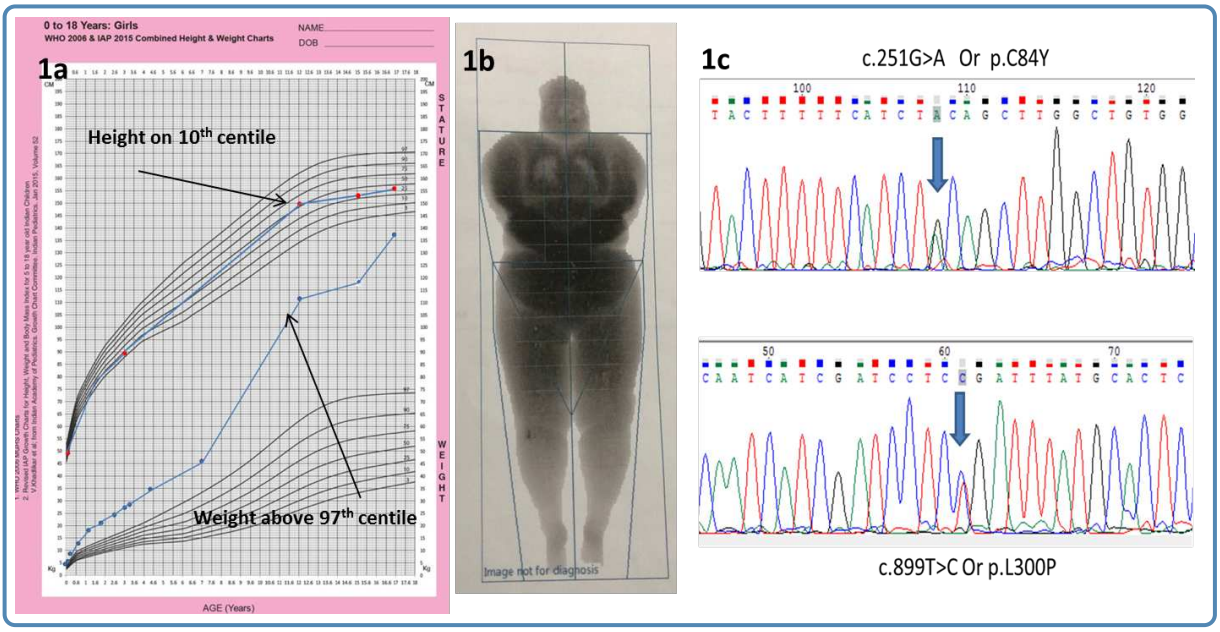

Figure 1 1a. Growth chart of the patient showing weight above 97th centile and height on 10th centile; 1b. Dual-energy X-ray absorptiometry (DEXA) scan showing fat distribution of the patient; 1c. Sequence chromatograms showing compound heterozygous variants p.(Cys84Tyr) and p.(Leu300Pro).

DNA methylation test for *SNRPN* gene revealed normal results. Clinical exome sequencing in the patient showed compound heterozygous variants c.899T>C [p.(Leu300Pro); paternally derived; Chr18:58038684:A>G; 59x coverage] and c.251G>A [p.(Cys84Tyr); maternally derived; Chr18:58039332:C>T; 100x coverage] in the *MC4R* gene (transcript id ENST00000299766). Both the variants were predicted to pathogenic by the *in silico* prediction tools Sorting Tolerant from Intolerant (SIFT), Polyphen2 and MutationTaster software (Kumar et al., 2009; Ramensky et al., 2002; Schwarz et al., 2014). The c.899T>C variant has not been reported in the 1000 Genome and ExAC databases but has been reported in the gnomAD database with a minor allele frequency of 0.000008125; the c.251G>A is a novel variant. The *MC4R* gene variants were confirmed through Sanger sequencing in the proband and parents (Figure 1c). Mutation analysis could not be done for the brother.

Discussion

The patient was clinically suspected to have monogenic obesity since there was severe early-onset obesity and hyperphagia, leading to other comorbidities such as insulin resistance, hyperlipidemia, Blount disease and polycystic ovarian disease. The genetic test identified variants in *MC4R* gene that encodes a 332 amino acid Melanocortin 4 receptor, a seven-transmembrane c-AMP pathway linked protein, as causative of obesity in the child. Functional studies for the identified variants could not be done.

Published literature on patients with *MC4R* gene mutations and phenotypic characteristics was reviewed. In 1998, Yeo et al. and Vaisse et al. reported mutations in *MC4R* gene with severe early-onset obesity. Since then, around 369 unique variants have been identified in various families affected with *MC4R* gene defect with haploinsufficiency as the predominant mechanism and dominant negative effect for some variants (Morell-Azanza et al., 2019). Certain variants may also be population specific. Thearle et al. (2012) found that the p.Arg165Gln and p.Asp37Ter mutations were present in 1.8 % full heritage Pima Indians in Arizona explaining the high prevalence of obesity and diabetes in that population. The *MC4R* gene defect is considered to be the commonest single gene cause of obesity with most studies reporting a prevalence of 1 to 6 % in patients with severe obesity. The

inheritance is dominant, with individuals with mono-allelic variation being less severely affected than the bi-allelic variations. The gene plays an important role in the leptin-melanocortin pathway that controls food satiety and energy expenditure. The adipocyte generated leptin hormone acts on the leptin receptor in the arcuate nucleus which releases the alpha melanocyte secreting hormone (MSH). MSH activates the *MC4R* receptor which is a G-protein coupled transmembrane receptor expressed in the paraventricular nucleus resulting in a satiety signal.

Kobayashi (2002) described a child with homozygous p.Gly98Arg variant in *MC4R* with weight gain from 10 months of age with BMI of 62 kg/m2 at 40 years of age. She had normal puberty. Bone mineral density was high (lumbar Z score 2.02). Insulin and leptin were elevated (4x and 5x of upper normal limit). Children with monoallelic mutations show greater weight gain rate during childhood but stabilized BMI in adulthood. Patients have higher linear growth with around 1.5 cm gain in adult height for partial loss of function mutations and around 5 to 7 cm gain for complete loss of function mutations (Martinelli et al., 2011; Thearle et al., 2012). Patients with monoallelic dominant negative mutations have severe early-onset obesity (Biebermann et al.,2003).

Hyperphagia may abate in adolescents and some may develop periods of anorexia nervosa leading to rapid and significant weight loss with rebound effects. Presence of obesity in index cases can also lead to "obesogenic" influence on non-carrier sibs thereby causing obesity in them.

Treatment options are limited and are based on case reports rather than randomised controlled trials. Dietary and exercise regimens are important but long-term efficacy is poor as reported in most patients with biallelic mutations. Hainerova and Lebl (2013) reported efficacy of sibutramine (serotonin and noradrenaline reuptake inhibitor) in one patient with biallelic *MC4R* mutations. Efficacy of glucagon-like peptide-1 receptor agonist liraglutide was evaluated and found to cause a 6% weight loss in 16 weeks in patients with *MC4R* heterozygous mutations and in those without. We did not find weight loss in our patient after three months of liraglutide therapy and rather weight gain was documented. There exists a debate about use of bariatric surgery in patients with *MC4R* gene variations. The Genetics of Obesity Study Consortium (GOOS), United Kingdom (https://www.mc4r.org.uk/) reported that patients with *MC4R* gene biallelic variations may not respond to bariatric surgery. This was based on a study by Hatoum et al. (2012) wherein 972 patients undergoing Roux en Y gastric bypass surgery (RYGB) were studied for *MC4R* gene variations. They found that patients with heterozygous *MC4R* variant responded equally to surgery as those without. They also reviewed that till 2011 less than 10 biallelic *MC4R* gene mutation carriers were reported and none of them had undergone RYGB. Aslan et al. (2011) performed laparoscopic adjustable gastric banding and truncal vagotomy for a 19 years old girl with *MC4R* gene mutations with BMI of 54.27 which resulted in initial weight loss but after 12 months, her BMI was 56.39. Bariatic surgery has been successfully practiced in adolescents with severe obesity and meeting the criteria of International Pediatric Endosurgery group (https://www.ipeg.org/morbidobesity). Teen Labs Consortium (Inge et al, 2011) reported the outcome of 242 adolescents (mean age 17.1) with mean BMI of 50.5. Mean weight loss sustained at end of three years was 28 % after gastric bypass and 26 % in those undergoing sleeve gastrectomy. However, mutation profile was not analysed. We have recommended Roux-en-Y gastric bypass for our patient based on these observations and also due to presence of co-morbidities such as Blount disease, polycystic ovarian disease and hyperinsulinism not responding to long-term dietary management.

Conclusion

Biallelic *MC4R* variations lead to severe early-onset obesity refractory to diet and exercise regimens, liraglutide treatment and early gastric bypass surgery. Affected individuals should be evaluated to prevent co-morbidities such as hyperinsulinism, joint disease, cosmetic issues/body image and psychological issues.

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