Melanocortin 4 receptor (MC4R) gene variants in children and adolescents having familial early-onset obesity: genetic and clinical characteristics

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Abstract
Melanocortin 4 receptor gene plays an important role in food intake, energy balance, and weight control. The autosomal dominantly inherited MC4R variants cause obesity by causing hyperphagia and decreased sense of satiety. Homozygous variants are rarely reported, and they cause earlier/severe obesity. Our objective is to determine the MC4R gene variant frequency in children and adolescents with familial early-onset obesity. One hundred thirty-nine children and adolescents (57 girls/82 boys) whose weight increase started before the age of 5 years and who had early-onset obesity in at least one of their first-degree relatives were included in the study. Obesity is defined as body mass index (BMI) of ≥95th percentile, and as extreme obesity is defined if the BMI ≥120% of the 95th percentile or ≥35 kg/m². Children having genetic syndromes associated with obesity and mental retardation or taking drugs that promote changes in eating behavior or weight were excluded from the study. Coding region of the MC4R gene was sequenced by using the Illumina MiSeq Next Generation Sequencing System. The mean age of the patients was 7.3 ± 3.7 years, and the mean BMI SDS was 3.7 ± 0.7. While 118 patients (85%) were prepubertal, 21 patients (15%) were pubertal. Seven different variants were identified in 12 patients by giving a variant detection rate of 8.6%, of these five were previously identified missense variants p.N274S, p.S136F, p.V166I, p.R165W, and p.I291SfsX10. One homozygous variant p.I291SfsX10 (c.870delG) was detected in a severely obese 2-year-old boy, and other variants were heterozygous. Two novel variants were found: p.M200del and p.S188L. By using the in silico analysis software, these novel variants were predicted to be disease causing.
Conclusion: MC4R gene variants are quite common in childhood obesity in Turkish population. Screening the variants in MC4R gene is necessary in patients with severe childhood-onset obesity. In such patients, comorbidities of obesity can be seen from early years.

What is known
• The frequency of MC4R mutations in obese patients was approximately 0–6.3%.

What is new
• In obese Turkish pediatric population, unlike other European countries, MC4R gene variants are quite common as we found a variant rate of 8.6%.
• We believe it is necessary to screen the variants in MC4R gene in patients with severe childhood-onset obesity and who had early-onset obesity in at least one of their first-degree relatives in Turkish population.

Keywords Monogenic obesity · Pediatrics · MC4R

Abbreviations
BMI Body mass index
HOMA-IR Homeostasis model assessment-insulin resistance
MC4R Melanocortin 4 receptor
SDS Standard deviation score
TSH Thyroid-stimulating hormone

Introduction

Obesity is a worldwide epidemic with rates nearly doubling over the last 30 years [1]. Although the major driving cause behind obesity is overeating, there is a considerable evidence of a significant genetic contribution for regulation of body weight. In 1998, MC4R variants were reported to be associated with dominantly inherited human obesity [2].

MC4R codes a protein called melanocortin 4 receptor, which is mainly found in the hypothalamus and is responsible for controlling appetite and satiety. It encodes the MC4R protein, a G protein-coupled receptor that binds the α-melanocyte-stimulating hormone (α-MSH). In murine models, MC4 receptors have been found to be involved in the feeding behavior, regulation of metabolism, sexual behavior, and male erectile function [3]. In animal models, deletion of MC4R also results in hyperphagia and increased body fat, ultimately leading to hepatic steatosis without atherogenic diet [4].

Since the first variants in MC4R in obese humans were reported over 20 years ago, several groups have reported the sequence variants in MC4R in different populations. Based on the studies and observations, MC4R variants seem to have an incomplete penetrance and some degree of codominance. Individuals that carry pathogenic variants have a 4.5-fold increased risk of developing obesity when compared with noncarriers [5].

There is a large variation in the frequency of variants between different studies ranging from 0.5 to 8.5% [2, 6]. More than 200 variants have been identified to date, primarily heterozygous dominant acting missense variants [7]. Heterozygous variants are also found in 2–5% of subjects with extreme pediatric obesity, making this the most common genetic form of obesity in pediatric age group [8, 9]. Homozygous MC4R variants have also been identified in offsprings from consanguineous families [9, 10].

In this study, the screening of the MC4R coding sequence of Turkish obese children and adolescents who have early-onset obesity was described. The aims of the present study were (i) to determine the frequency of MC4R variants in a cohort of Turkish clinically obese children and adolescents and (ii) to search for variants in the promoter region of MC4R. In addition, a list of all variants described in the literature, which will aid the interpretation of variants found in a diagnostic setting, was provided.

Material and method

One hundred thirty-nine children and adolescents (57 girls/82 boys) whose weight increase started before the age of 5 years, who presented to Pediatric Endocrinology and Medical Genetics Department of Ege University Medicine Faculty, and who had the history of early-onset obesity in at least one of their first-degree relatives were included in the study. Obesity is defined as body mass index (BMI) of ≥ 95th percentile, and as extremely obese is defined if the BMI ≥ 120% of the 95th percentile or ≥ 35 kg/m2 [11]. Children having genetic syndromes associated with obesity and mental retardation or taking drugs that promote changes in eating behavior or weight were excluded from the study. Coding region of the MC4R gene was sequenced by using the Illumina MiSeq Next Generation Sequencing System.

Physical examinations of the cases were performed by an experienced pediatric endocrinologist and were recorded in the patient data form. Height was measured as the nearest 0.5 cm by stadiometer. Body weight was measured using an electronic scale sensitive to the nearest 100 g. BMI was calculated as kg/m². Body weight, height, and BMI’s SD scores were calculated using Turkish national anthropometric references [12]. Blood pressure values above 95th percentile according to age, sex, and height were accepted as hypertensive [13]. Blood glucose, insulin, and serum lipid levels measured at admission during...
morning fasting were recorded from the file data. Homeostasis model assessment that shows the insulin resistance is calculated by the following formula: insulin resistance (HOMA-IR) value = fasting blood glucose (mg/dl) × 0.055 × fasting insulin (mIU/mI)/22.5. In a systemic review including 8732 children and adolescents, the value of HOMA-IR associated with metabolic syndrome ranges from 2.30 to 3.54 [14]. The cutoff value of 3.16 was chosen in line with previous studies in obese children and adolescents [15–18]. Written informed consents were obtained from all the participants. This study was approved by the Ege University Ethics Committee.

Genetic analysis

Molecular diagnosis of DNA was isolated from a 200-μl blood sample using the QIAamp DNA Blood Mini QIAcube Kit and a QIAcube instrument (QIAGEN, Hilden, Germany) according to the manufacturer’s specifications. The entire coding sequence of MC4R gene (NM_∗155541) was PCR amplified and sequenced on Illumina MiSeq System using 300-cycle Reagent Kit V2. Base-calling and sequence alignment were performed by using the built-in MISEQ 4 REPORTER software.

The primers used were MC4R-F (5’T-ATCA ATTCAGGGGGACACTG 3′) and MC4R-R (5’-GGCC ATCAGGAACATGTGGA-3′) for MC4R gene sequencing.

All variants in MC4R gene with a frequency of less than 0.5% in public databases (e.g., NCBI dbSNP build141 [19], 1000 Genomes Project [20], Exome Aggregation Consortium (ExAC) [21], NHLBI Exome Sequencing Project (ESP), and Exome Variant Server [22]) were selected. The prediction of the potential damaging effects of variants on protein activity with different algorithms was identified using several in silico prediction tools such as MutationTaster [23] and SIFT [24]. The variants were evaluated by VarSome [25], evolutionary conservation scores [24] determined, and variants categorized in accordance with the ACMG recommendations [26].

Statistical analysis

The statistical analysis of the data was carried out by using SPSS 21.0 (Chicago, IL, USA). Mann-Whitney U-test and chi-square test were used to compare numerical and categorical variables, respectively. A p value of < 0.05 was accepted to represent statistical significance. The data were presented as mean ± SD or n (%).

Results

The average age of admission of 139 cases included in the study was 7.3 ± 3.7 years (between 1.3 and 15 years), mean height SDS was 1.4 ± 1.1, mean BMI was 39.2 ± 8.9 kg/m², and mean BMI SDS was 3.6 ± 0.7 SD. While 118 (85%) cases were in the prepubertal period, 21 (15%) cases were in the pubertal period. Age of obesity onset was found as 3.2 ± 2.1 years in the study group. Mean birth weight was 3546 ± 746 g.

Seven different variants in 12 patients were identified by giving a variant detection rate of 8.6%. Of these, 5 were previously identified missense variants p.N274S, p.S136F, p.V166L, p.R165W, and p.I291SfsX10. Previously identified p.N274S variant was the most common detected variant and was found in 4 cases from different families. Two novel variants were found: p.M200del and p.S188L. By using the in silico analysis software, these novel variants were predicted to be disease causing (Table 1).

Age of obesity onset was found to be lower in the variant carrier cases (2.2 ± 1.1 vs 4.7 ± 2.9 years, p = 0.01). No statistically significant difference was found between the cases with and without variant in terms of age, height SDS, BMI, BMI SDS, blood pressure, serum fasting glucose, and lipid values. HOMA-IR value was higher in variant carrier group (5.4 ± 1.8 vs 3.9 ± 2.6, p = 0.04) (Table 2). No significant difference was found between the groups in terms of blood pressure and serum lipid levels.

In variant carrier group, mean BMI SDS was found as 3.8 ± 1.5 SDS. While insulin resistance was found in 11 (91.6%) cases, acanthosis nigricans was found in 3 (25%) cases, liver steatosis in 5 (41.6%) cases, psychiatric disorder in 2 (16.6%) cases, and TSH elevation in 2 (16.6%) cases. High blood pressure was observed in only one case. Diabetes was not detected in any patient.

Homozogous p.I291Sfs*10 variant causing the formation of stop codon resulting in frameshift was detected in 2-year-old male patient (patient 5), and he had increased appetite and weight increase from the sixth month of his life. BMI SDS value of this case was 7.3 SD (severe obesity). The patient’s mother was shown to have a heterozygous variant (mother’s BMI SDS, 1.5 SD), and no sample could be taken from the father. It was decided that the variant was pathological because variant caused the stop codon and had a correlation with the disease in in silico programs.

One patient (patient 12) admitted to us with severe obesity had p.N274S variant, and he had also hepatosteatosis, thyroid-stimulating hormone (TSH) elevation (5.19 mIU/L), systolic and diastolic non-dipper hypertension, and insulin resistance. During follow-up due to rapid weight gain and major depressive disorder, sleeve gastrectomy operation was performed in 16 years old (175 kg + 5.64 SDS; 194 cm + 3.15 SDS; BMI, 46.5 + 3.76 SDS). Table 1 shows the clinical data, comorbidities, and genetic characteristics of the cases with MC4R gene variants.

Discussion

Heterozygous MC4R variants have been reported in obese people from various ethnic groups. The prevalence of
| Case number | Sex/age (years) | Age at onset of obesity (years) | Height SDS/BMI SDS | Comorbidity | HOMA-IR | cDNA | Protein | ACMG/AMP | Mutation type | MT | SIFT | GERP | ExAC* (overall allele frequency) | Novel |
|-------------|----------------|---------------------------------|--------------------|-------------|---------|------|---------|----------|--------------|-----|------|------|----------------------------|-------|
| 1           | M/10           | 3                               | 1.9/3.7            | AN, IR      | 6.3     | c.821 A > G/wt | p.N274S/wt | P | MS | DC | D | 5.8499 | 0.00001647 | – |
| 2           | F/8.6          | 2                               | 1.9/2.7            | IR, hepatosteatosis | 4.4     | c.496 G > A/wt | p.V166I/wt | UP | MS | DC | D | 5.8499 | 0.00000879 | – |
| 3           | M/8.5          | 2                               | 3.2/4.6            | IR, depression, social isolation | 8.1     | c.496 G > A/wt | p.V166I/wt | UP | MS | DC | D | 5.8499 | 0.00000879 | – |
| 4           | M/14           | 1                               | 0.8/3.6            | IR, hepatosteatosis, TSH elevation | 7.3     | c.407 C > T/wt | p.S136F/wt | UP | MS | DC | D | 5.6999 | – | – |
| 5           | M/2            | 0.6                             | 1.8/7.3            | IR          | 3.2     | c.870delG/c.870delG | p.I291Sfs*10/ | LP | fs | DC | NA | 6.0599 | – | – |
| 6           | F/8            | 4                               | 1.2/2.9            | AN, IR      | 3.4     | c.821 A > G/wt | p.N274S/wt | P | MS | DC | D | 5.8499 | 0.0000176 | – |
| 7           | F/14           | 2                               | 1.0/3.1            | IR, hepatosteatosis | 4.6     | c.407 C > T/wt | p.S136F/wt | US | MS | DC | D | 5.6999 | – | – |
| 8           | M/14.5         | 3                               | 1.1/3.0            | IR          | 6.2     | c.821 A > G/wt | p.N274S/wt | P | MS | DC | D | 5.8499 | 0.00001647 | – |
| 9           | M/2.5          | 0.5                             | 1.8/3.1            | None        | 0.8     | c.563C > T | p.S188L/wt | US | MS | DC | D | 5.8499 | 0.00000879 | + |
| 10          | M/13.3         | 3.5                             | 1.4/3.2            | IR, hepatosteatosis | 4.6     | c.493C > T | p.R165W/wt | US | MS | DC | D | 5.8499 | 0.0000176 | – |
| 11          | M/11.7         | 4                               | 0.4/2.9            | IR          | 3.7     | c.597_599delCAT | p.M200del/wt | US | del | – | – | 5.8499 | – | + |
| 12          | M/16           | 5                               | 2.8/3.8            | IR/AN/hepatosteatosis/ hypertension/TSH elevation | 8.1     | c.821 A > G | p.N274S/wt | P | MS | DC | T | 5.8499 | 0.0000176 | – |

*Exome Aggregation Consortium (http://exac.broadinstitute.org). The allele frequency in the ExAC database does not contain representative controls for all ethnic groups.

M male, F female, MS missense, NS nonsense, del deletion, fs frame shift, MT MutationTaster, DC disease causing, PD probably damaging, D damaging, T tolerated, NA not available, wt wild type, P pathogenic, LP likely pathogenic, US uncertain significance, SIFT sorting intolerant from tolerant, AN acanthosis nigricans, IR insulin resistance.
pathogenic \(MC4R\) variants varies between 0.5 and 8.5% of obese adult and pediatric patients [27]. Single-nucleotide polymorphisms in \(MC4\) gene are also related to obesity and its metabolic complications [28]. Previously, Demiralp D et al. [29] studied \(MC4\) gene polymorphisms in obese Turkish children. In their study, they showed that p.V103I polymorphism was 4.5% in complicated obese children and p.E42K polymorphism was 9% in familial obese children and 1.5% in complicated obese children. De Rosa et al. [30] study 312 African American and Latino children with severe non-syndromic obesity, and their variant rate was 2.6%. In the recent study by Tunç et al. [31], \(MC4\) gene variant was investigated in 47 morbid obese children. This study composed of the cases that have the same ethnic origin, and the variant rate was given as 8.5% that is very similar to the present study [28]. Aküncü et al. [32] study 105 patients with severe (BMI > 3) early-onset obesity for 41 previously known obesity-related genes by targeted next-generation sequencing analysis, and they found monogenic obesity of 10.4% in Turkish population. In our study, nucleotide sequence of the coding region of \(MC4\) was determined in 139 unrelated probands with familial early-onset obesity, and 8 (8.6%) probands with variants that alter the amino acid sequence of the receptor were found.

Beckers S et al. [33] reported the frequency of polymorphism in a cohort including 123 obese children and adolescents as 3.25%, and they did not detect pathogenic \(MC4\) variant in any case. However, in their study, the sample group consisted of only obese patients, and it did not discriminate any early-onset obesity. Farooqi et al. [10] examined the nucleotide sequence of the \(MC4\) gene in 500 individuals with severe childhood-onset obesity. Of them 29 individuals (5.8%) had pathogenic variants in \(MC4\): 23 were heterozygous and 6 were homozygous. The reason of these frequency differences in the literature may be due to different inclusion criteria of studies or different ethnic origins.

Homozygous or compound heterozygous \(MC4\) variants are associated with more severe obesity than the heterozygous form, revealing a codominant mode of inheritance [34]. Fewer than 20 cases of homozygous, \(MC4\) variants have been reported in the literature. In the present study, one patient’s extreme obesity was secondary to homozygous p.I291Sfs*10 variant in \(MC4\) gene. In the study of Tunç et al. [28], BMI SDS of 6-year-old index case having heterozygous form of the same variant was given as 3.01. On the other hand, in the present study, the case having homozygous variant was 2 years old and severely obese with BMI SDS of 7.3 at an earlier age.

In patients with \(MC4\) variants, insulin resistance is expected from the early years of life due to hyperphagia and early-onset obesity. In the present study, it was found that 91.6% of the cases with \(MC4\) variant had insulin resistance that was proven by the laboratory. HOMA-IR values were statistically higher in cases with variant compared with the cases without variants. Even though Tunç et al. [31] reported that there was no insulin resistance in their study group, fasting insulin values were median 22.4 ± 7.5 mIU/L, high for age references (0–17 mIU/L). Previous studies also reported the presence of insulin resistance in cases with \(MC4\) variants [10, 35]. In 1362 Indian children, \(MC4\) rs12970134 polymorphism is also related to increased insulin resistance [36]. Within the variant carrier cases in the present study, insulin resistance was determined as early as 2 years. For this reason, cases with \(MC4\) variant should be monitored for insulin resistance and related complications from earlier age.

**Table 2** Comparison of clinical and laboratory findings between \(MC4\) variants detected and undetected patients

| Features                  | \(MC4\) (-) n (127) | Mean ± SDS | \(MC4\) (+) n (12) | Mean ± SDS | \(p\)   |
|---------------------------|---------------------|------------|-------------------|------------|---------|
| Age (years)               | 8.1 ± 2.3           | 7.8 ± 5.2  | 0.17              |
| Age of onset of obesity   | 4.7 ± 2.9           | 2.2 ± 1.1  | 0.01              |
| Height SDS                | 1.4 ± 0.9           | 1.2 ± 1.4  | 0.22              |
| BMI (kg/m²)               | 37.1 ± 10.5         | 42.2 ± 9.9 | 0.15              |
| BMI SDS                   | 3.6 ± 0.8           | 3.8 ± 1.5  | 0.16              |
| Systolic blood pressure (mmHg) | 97.3 ± 32.0       | 93.9 ± 21.1 | 0.45              |
| Diastolic blood pressure (mmHg) | 64.4 ± 28.0       | 62.7 ± 26.3 | 0.57              |
| Fasting glucose (mg/dL)   | 85.6 ± 10.7         | 89.9 ± 10.2 | 0.54              |
| Fasting insulin (mIU/ml)  | 17.8 ± 11.9         | 20.2 ± 11.9 | 0.21              |
| HOMA-IR                   | 3.9 ± 2.6           | 5.4 ± 1.8  | 0.04              |
| HbA1c (%)                 | 5.4 ± 0.5           | 5.5 ± 0.7  | 0.66              |
| Total cholesterol (mg/dL) | 189.3 ± 78.1        | 191.7 ± 82.1 | 0.76              |
| Triglycerides (mg/dL)     | 153.2 ± 66.2        | 167.1 ± 62.6 | 0.88              |
| HDL (mg/dl)               | 41.6 ± 9.8          | 40.2 ± 10.2 | 0.97              |

\(MC4\) melanocortin 4 receptor, SDS standard deviation score, HOMA-IR homeostasis model assessment-insulin resistance, BMI body mass index, HDL high density lipoprotein.
There are a limited number of studies showing the correlation between MC4R and hypothalamic-pituitary-thyroid axis. While serum free T4 levels were normal in all cases in the sample group, elevation of isolated TSH was observed in two cases, and TSH values were between 5.2 and 7.3 mIU/L in the follow-up of a case with normal autoantibody levels, thyroid ultrasonography, and urine iodine excretion. No decreased in free T4 or apparent hypothyroidism was observed. In the previous study by Farooqi S et al. [10], isolated TSH elevation was reported in 1 of 29 patients with MC4R variant. In the study conducted by Huszar D et al. [37] on MC4R knockout mice, no correlation between the MC4R molecule and the thyroid axis was found. Vella KR et al. [38] showed in their study that mice with neuropeptide-Y and MC4R deficiency had impaired thyrotropin-releasing hormone, TSH, and thyroid hormone suppression in hypothalamic-hypophyseal areas and also neuropeptide-Y and MC4R were required for the hepatic metabolism of T4. Further studies are needed regarding the effect of the MC4R molecule on the hypothalamic-pituitary-thyroid axis, which also has an effect on energy metabolism.

A significant correlation has been reported between obesity/overweight and some psychiatric disorders especially attention deficit hyperactivity disorder and depression. This suggested that these two conditions might share common molecular pathways despite their heterogeneity. However, the pathogenesis of these correlations is not known clearly [39, 40]. Agranat-Meged A et al. [40] suggested that attention deficit hyperactivity disorder was statistically significantly more frequent in the cases with C271R variant in the MC4R gene. In their study, Mergen et al. [41] showed a bipolar psychiatric disorder in a female patient who had p.N274S heterozygous variant. In the current series, a 8.5-year-old male patient with p.N274S variant and morbid obesity also had major depressive disorder, and he was hospitalized in an adolescent psychiatric clinic due to major depression. Whether these two conditions may be secondary to obesity, separate clinical entity, or secondary to variant in MC4R gene, it was not yet fully explained [42–44]. Further studies are required in this field.

Moreover, plenty of pharmacological studies are ongoing in obesity treatment. Setmelanotide (MC4R agonist) treatment in MC4R variant carriers was investigated in a randomized, double-blind, placebo-controlled Phase Ib study. After 28 days, a mean difference in weight loss of 0.6 kg/week was observed in MC4R variant carriers compared with the placebo subtracted group [45]. As a result, detection of variant carrier patients may also be important for the chance of treatment in the near future.

In conclusion, MC4R gene variants are quite common in childhood early-onset obesity in Turkish population. It is necessary to screen the variants in MC4R gene in patients with severe childhood-onset obesity. Cases with MC4R variants should be closely followed up for obesity complications and comorbidities from early ages.

**Authors’ contribution** A.A: Methodology, conceptualization, investigation
S.O: Software, investigation, writing, original draft preparation
D.G: Investigation
A.A: Investigation, writing
H.O: Methodology, software
T.A: Formal analysis, investigation
S.D: Project administration
F.O: Writing, reviewing, editing

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**Compliance with ethical statements**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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