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CASE REPORT

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Identification of p.Met215Ile mutation of the *MC4R* gene in a Moroccan woman with obesity

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Abstract

Screening the *MC4R* gene showed one rare mutation p.Met215Ile in a Moroccan patient with morbid obesity, which leads to a change in the protein structure. The analysis of *MC4R* variants may be useful for future therapeutic approaches.

KEYWORDS

human genetics, in silico analysis, *MC4R* gene, mutation, obesity

1 | INTRODUCTION

The *MC4R* gene is involved in the leptin-melanocortin pathway which mutations can lead to severe forms of obesity. Here, we report one rare mutation p.Met215Ile in a woman with morbid obesity. This mutation leads to changes in protein structure through the loss of two hydrophobic interactions, and it is predicted to be disease-causing.

The *melanocortin-4-receptor gene* (*MC4R*) plays an important role in energy homeostasis, food intake, and body weight regulation.[1] The genetic variants of the *MC4R* coding region are associated with common and severe forms of obesity, mainly characterized by hyperphagia starting from the first years of life.[2]

The MC4R is a protein of 332 amino acid belonging to the family of seven transmembrane (TM) spanning G-protein coupled receptors (GPCRs) that binds the α-melanocyte-stimulating hormone (α-MSH).[3] It is expressed in the paraventricular nucleus of the hypothalamus and plays an important role in the regulation of food intake and body weight through the melanocortin pathway. It is stimulated by circulating leptin (LEP) which is secreted by adipose tissue.[4] The MC4R binds to its ligand α-MSH and induces anorexigenic effects. This ligand binding activates G proteins to direct signaling and gene transcription, a response that is diminished within minutes when phosphorylated MC4R bind to β-arrestin, which sterically prevent its coupling to G proteins.[5] Classically, the receptor coupling results in the activation of phospholipase C (PLC) and increased cytosolic calcium concentration through Gαq which results in decreased food intake.[6] The α-MSH also increases energy expenditure by regulating intracellular concentrations cyclic adenosine monophosphate (cAMP) production by increasing adenylyl cyclase (AC) activity through Gαs signaling,[7] followed by activation of protein kinase A (PKA), exchange protein

activated by cAMP (EPAC), extracellular signal-regulated kinase 1/2 (ERK1/2), C-AMP Response Element-binding protein (CREB), and increased transcription of c-Fos and decreased AMP-activated protein kinase (AMPK).[8,9] The α-MSH signaling also increases firing in MC4R neurons by activity regulation of the potassium channels.[10] The ligand binding results in closure of Kir7.1 leading to depolarization of MC4R neurons and playing a central role in the regulation of energy homeostasis.[11] The Gαs mediated also the recruitment of β-arrestin resulting in the internalization and desensitization of the receptor.[5] In fact, its endocytosis and targeting to early endosomes followed by rapidly recycling to the cell membrane or translocate to lysosomes for degradation.[8] The arrestin could also contribute to melanocortin signaling. It induces activation of the MAPK pathway leading to phosphorylation of ERK1/2.[12]

The *MC4R* gene has a single exon on chromosome 18q21.32. The mutations of this gene are responsible for 2% to 3% of obesity in adults and children,[13] and the inheritance is autosomal codominant with incomplete penetrance.[14] The subjects that carry pathogenic variants have a twofold to ninefold increased risk of obesity when compared with non-carriers.[15] More than 200 mutations have been described[11] in European, American and Asian populations.[16–26] The majority of the mutations of *MC4R* are heterozygous missense.[13] The carriers of homozygous or compound heterozygous mutations are rare and only a few individuals have been described.[14,27–30] These patients exhibited morbid obesity than their heterozygous relatives.[14]

The objective of our study was to identify the rare monogenic variant of the *MC4R* gene in a cohort of Moroccan women with obesity.

2 | PATIENTS AND METHODS

2.1 | Participant recruitment

The study cohort comprised unrelated Moroccan women with a body mass index (BMI) ≥30 kg/m^2 from the Clinical Nutrition Unit of Military Hospital Mohammed V in Rabat, Morocco. Pregnant, breastfeeding, menopausal patients, and those with known diseases or on drug treatments inducing secondary obesity were not included. The BMI (kg/m^2) and the body fat mass (BFM) (%) were evaluated. The family history and age at onset of obesity were reported. We also noted known disorders from the medical records of the subjects. We collected 3 ml of peripheral blood from patients after obtaining informed consent from January to December 2018.

The protocol of this study was approved by the Ethics Committee of the Faculty of Medicine and Pharmacy in Rabat, Morocco.

2.2 | Screening of *MC4R* gene

The DNA was extracted from the peripheral blood using the phenol-chloroform method and stored at +4°C. Thereafter, the *MC4R* exon was amplified by primer sets designed using the primers3plus software in the Biometra thermal cycler (Table 1). The reactions were performed in a total volume of 25 μl, containing 10X PCR buffer, 2.5 mM of $MgCl_2$, 0.5 mM of dNTPs, 0.5 μM of each primer, and 0.75 Units of Taq DNA Polymerase (Applied Biosystems). The amplification consisted of initial denaturation at 94°C for 5 min, and 39 cycles of 30 s denaturation at 95°C, 30 s annealing at a temperature depending on the melting temperature (Tm) of primers, and 30 s at 72°C followed by a final extension at 72°C for 7 min. The PCR products were visualized on 2% agarose gels. The PCR and sequencing products were purified by gel filtration through Sephadex G-50 superfine.

The PCR products were sequenced using BigDye Terminator version 3.1 (Applied Biosystems) in the Biometra thermal cycler. The reactions were performed in a final volume of 11 μl, containing PCR products, 5X sequencing buffer, 0.5 μl BigDye, and 10 μM of primer. The sequencing method was performed by an initial denaturation of 5 min and a program of 25 cycles of 94°C for 10 s, 52°C for 5 s, and 60°C for 2:30 min.

TABLE 1 Sequence and Tm of primer pairs for PCR

Gene	Primer	Primer (5′→3′)	PCR product size (bp)	Tm (°C)
MC4R	MC4R-I	AAACTCCATGTCAAGCTCTGG CTTGGCTATTGCCACAATCA	379	66.5
	MC4R-II	TGACTCTGGGTGTCATCAGC GCAAGCTGCCCAGATACAA	377	68.0
	MC4R-III	ATGACAGTTAAGCGGGTTGG CCCTTCATATTGGCACCTTG	248	62.0
	MC4R-IV	CCAGGCTTCACATTAAGAGGA ACGGAAGAGAAAGCTGTTGC	441	66.5

Abbreviations: bp, base pair; Tm, melting temperature.

The sequencing method was carried on the Applied Biosystems 3500xL Genetic Analyzer based DNA analysis instrument using capillary electrophoresis technology with eight capillaries. The sequences were analyzed by the Sequencing Analysis Software version 5.4 and compared to the RefSeq (Accession number: NG_016441.1) available from Genbank.

2.3 | Bioinformatics prediction and structural analysis of *MC4R* mutation

To predict the possible impact of the mutation on MC4R protein function, we used Polymorphism Phenotyping version 2 (PolyPhen-2: http://genetics.bwh.harvard.edu/pph2/), Sorting Intolerant From Tolerant (SIFT: https://sift.bii.a-star.edu.sg/) and Predict SNP effect (PredictSNP: https://loschmidt.chemi.muni.cz/predictsnp/). The guidelines of the American College of Medical Genetics and Genomics (ACMG) were used to classify the mutation.

We used the 3D structure of the MC4R protein identified by Yu et al.[31] We submitted the FASTA sequence to the Local Meta-Threading Server (LOMETS) for modeling of missing amino acids in the crystal structure of the protein. The 3D variant structure was generated using PyMOL version 2.4.1, and the energy minimization for the native and variant structure was achieved using YASARA Force Field Minimization Server.

3 | RESULTS

In this study, the screening of the *MC4R* gene in Moroccan women with obesity was performed. Seventy-three patients were recruited for this work but nine cases declined to continue their participation. Out of 64 subjects with obesity, a patient had one heterozygous missense mutation resulting from the substitution of methionine by isoleucine at codon 215 (p. Met215Ile). No other mutation was detected in the *MC4R* gene. The electropherogram of this rare variant is shown in Figure 1. This mutation identified by the Sanger sequencing method was confirmed in both the sense and antisense strands.

The mutation p. Met215Ile was identified in 39-year-old young women with childhood-onset obesity at age of 6 years (according to her) (North African Arab descent). She did not take hormonal contraceptives or medication. On anthropometric examination, her current and maximum weight was 102.50 kg; height, 1.60 m; BMI, 40.03 kg/m^2; waist circumference (WC), 112 cm; hip circumference (HC), 134 cm; waist-hip ratio (WHR), 0.84; and BFM, 51.70%. Her blood pressure was 110/70 mmHg. The values of her biochemical parameters were normal. Table 2 illustrates anthropometric parameters of the patient.

The patient was diagnosed with sciatica due to significant excess weight. She complained of hyperphagia and had difficulty following the prescribed diets. She reported

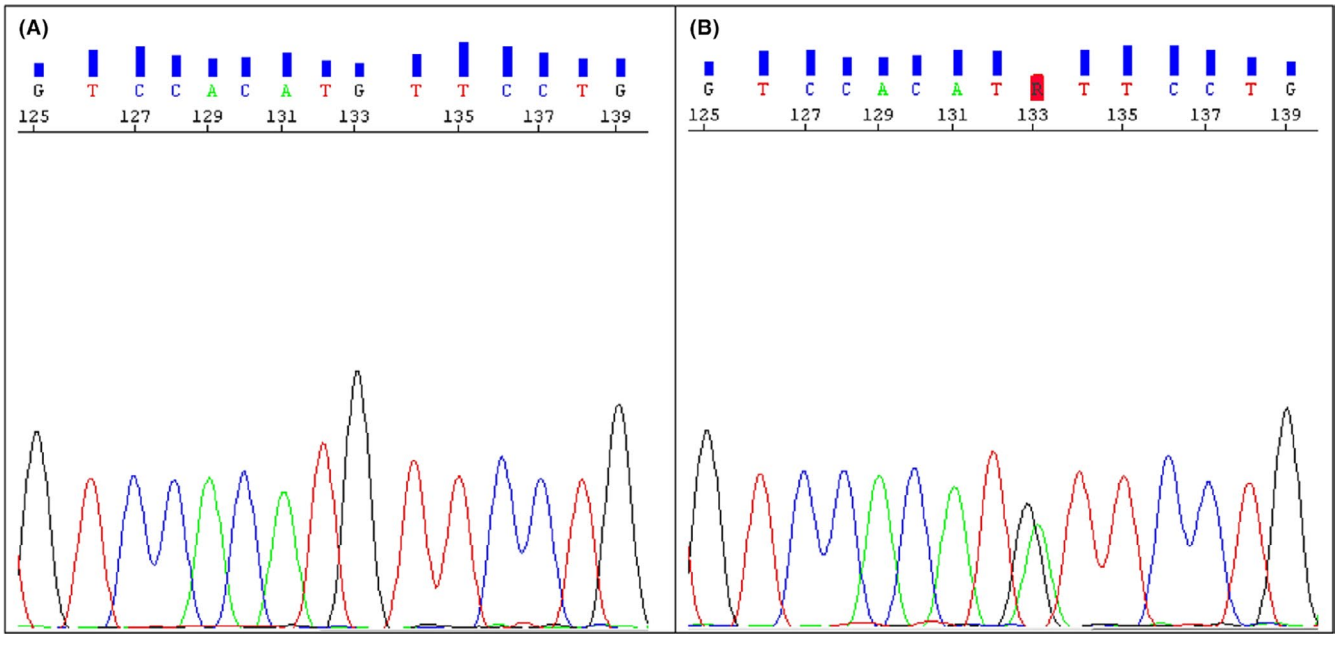

FIGURE 1 Electropherogram of *MC4R* variant identified in this study. Sequence electropherogram of a wild type (A) and a mutant (B) showing a heterozygous missense mutation in the *MC4R* gene resulting in the amino acid substitution from methionine to isoleucine at codon 215 (p. Met215Ile)

TABLE 2 Anthropometric parameters of the patient

Parameters	Values	Normal values
Age (years)	39	-
Weight (cm)	102.50	-
Height (cm)	1.60	-
BMI (kg/m^2)	40.03	18.50–24.99
Fat mass (%)	51.70	21.0–27.0
Waist circumference (cm)	112	⩽80
Hip circumference (cm)	134	-
WHR	0.84	⩽0.80

Abbreviations: BMI, body mass index; WHR, waist-hip ratio.

a family history of obesity (maternal grandmother, mother, and niece).

The effect of the p. Met215Ile was reported as probably damaging and deleterious by Polyphen-2, SIFT, and PredictSNP. The mutation was classified as likely pathogenic based on standards and guidelines of the ACMG.

We used prediction tools for structural analysis of p. Met215Ile variant. The three-dimensional structure of this variant is shown in Figure 2. The methionine at position 215 is located in the TM5 of the MC4R receptor (Figure 2c). In the wild type (Figure 2d), methionine at position 215 forms two hydrogen bonds with leucine at position 211 and alanine at position 219, and two hydrophobic interactions (one with histidine at position 214 and one more with alanine at position 244). In the mutant case (Figure 2e), the substitution of methionine by isoleucine at codon 215 (p.Met215Ile) induces the loss of all hydrophobic interactions.

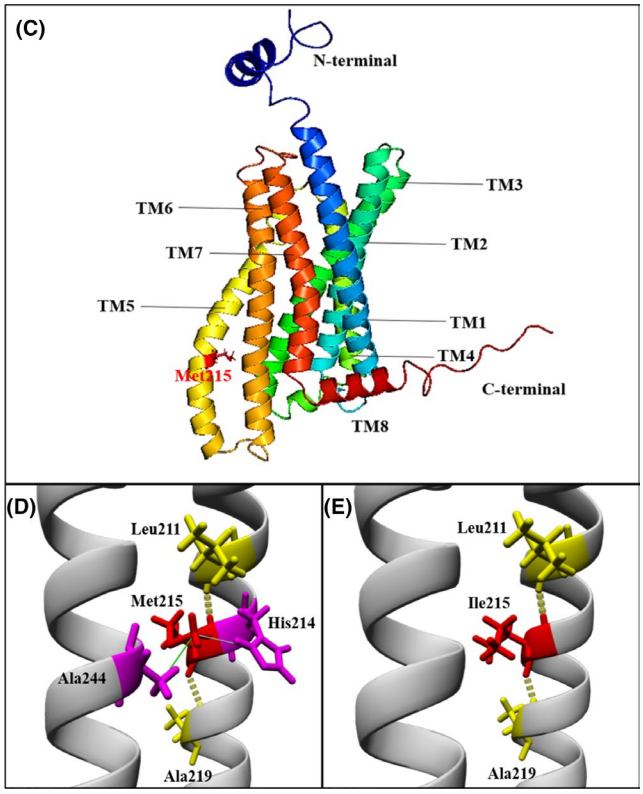

FIGURE 2 Three-dimensional effect of *MC4R* mutation p. Met215Ile. The tertiary structure of MC4R protein (C), 3D model of wild type (D), and mutant protein having a mutation from methionine to isoleucine at position 215 (E). The transmembrane (TM) helices are represented by different colors. The N-terminal domain is colored in dark blue and the C-terminal domain in red. The methionine at position 215 is colored in red and located in the TM helix 5. Residue substituted is showed in red, residues involved in hydrogen bonds are marked in yellow, residues that participate in hydrophobic interactions are indicated in purple. The substitution of methionine per isoleucine causes a loss of hydrophobic interactions

4 | DISCUSSION

In this study, we analyzed the coding region of the *MC4R* gene in Moroccan women with obesity. It is involved in the leptin-melanocortin pathway which mutations can lead to severe forms of obesity. We identified a rare mutation, p. Met215Ile (rs768687497), in a patient (1.56%) with morbid obesity (BMI = 40.03 kg/m^2) and an abnormally great desire for food. None of the 63 subjects evaluated carried any mutation in the *MC4R* gene. Furthermore, the p. Met215Ile was not found in 96 healthy Moroccan individuals,[32] nor in 333 healthy Moroccan controls (data not published yet). This variant is reported in 2/251.254 heterozygous alleles in individuals of non-Finnish European descent in GnomAD (AFNFE 0.000007960). It is also described in the Ensembl Genome Browser with a MAF <0.01.

In addition, the p. Met215Ile has been cited in subjects with obesity and underweight. This mutation has already been described in a boy with underweight and short normal stature in Germany[33] and in a 14-year-old girl with morbid obesity of Latino ancestry (BMI = 57.50 kg/m^2). This girl developed obesity in the first months of her life and had hypertension, dyslipidemia, pre-diabetes, hyperinsulinemia, and extensive acanthosis.[34] Interestingly, De Rosa et al. also reported that there was no history of family consanguinity and the obesity is caused by de novo mutation.[34] Recently, this variant was also identified in one case from 5724 participants of a specific Avon Longitudinal Study of Parents and Children (ALSPAC) UK Birth Cohort.[35] All carriers were heterozygous for this rare mutation. This variant is considered codominant with incomplete penetrance and variable expressivity.[29,35] This explains the difference in phenotype between individuals carrying the mutation and could be caused by a combination of genetic and environmental factors.

The p.Met215Ile is located in the TM5 of the MC4R.[35] The TMs have been identified as essential for ligand binding and signaling pathways mediated by the MC4R receptor for the regulation of appetite and metabolism. In the *MC4R* gene, some mutations can induce reduced/abolished α-MSH binding or altered intracellular signaling cascades.[36] According to Herrfurth et al, the methionine at position 215 is located in a critical region of the helix arrangements, which is modified during the receptor activation and is needed for binding G-protein or arrestin.[33] Indeed, two major signaling conformations are adopted by the receptor, one of which couples almost exclusively to arrestin, while the other also couples effectively to G proteins.[37] A study reported that the TMs5/6 bundle could interact with arrestin through hydrogen bond contacts.[38]

In our analysis of the receptor structure, the p.Met215Ile induces a loss of two hydrophobic bonds and this could affect the receptor conformation. The effects of this missense variant were classified as probably damaging and deleterious by in silico software, and as likely pathogenic according to ACMG variant classification guideline. Previous functional studies showed that the p.Met215Ile reduced basal activity of the receptor, which may play an important role in the development of obesity. Some functional assays were performed to analyze the surface expression of the receptor, the response of cAMP to agonist binding, and the activation of ERK/MAPK signaling.[33–35] Herrfurth et al. reported that this variant reduced cell surface expression by 80% compared to the wild-type receptor.[33] The ligand-activated cAMP accumulation was reduced following binding to NDP-α-MSH (a synthetic analog of α-MSH) and required a 10-fold higher concentration of α-MSH compared to the wild-type receptor.[33–35] In fact, the mutation resulted in reduced MC4R activity. It induced a 39.6% decrease in maximal signaling compared to wild-type MC4R signaling.[33] Also, Wade et al. demonstrated that this variant showed impaired β-arrestin-2 coupling.[35] As we know, the β-arrestins are major regulators of MC4R-mediated signaling processes and play an important role in the regulation of energy homeostasis.

Recently, Wade et al. reported that p.Met215Ile is responsible for a partial loss of the MC4R function.[35] This rare mutation may probably affect the development of obesity by inducing a partial loss of MC4R function.

In conclusion, we analyzed *MC4R* gene which plays a major role in the leptin-melanocortin pathway. This is the first study conducted in Morocco. We detected one rare *MC4R* mutation leading to change in the protein structure and partially reduced MC4R function. The analysis of *MC4R* variants may be useful for future therapeutic approaches.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

MEF, JEB, and ND conceived the study. MEF, HB, and HG participated in the recruitment. MEF and JEB carried out the experiments. MEF and JEB analyzed data. ZE performed in silico analyses. MEF and JEB contributed to the interpretation of results and drafting of the manuscript. All authors have read and approved the final manuscript.

ETHICAL APPROVAL

This study was approved by the ethics committee of the Faculty of Medicine and Pharmacy in Rabat, Morocco.

CONSENT

Written informed consent for publication was obtained from all participants.

DATA AVAILABILITY STATEMENT

The data used and analyzed during this research are available from the corresponding author on reasonable request.

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