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A NOVEL MELANOCORTIN 4 RECEPTOR (MC4R) GENE MUTATION ASSOCIATED WITH MORBID OBESITY

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ABSTRACT: Mutations in the melanocortin 4 receptor gene (MC4R) are the most common cause of monogenic human obesity. As part of our ongoing project entitled 'Turkish Obesity Genome Study' we determined the nucleotide sequence of the entire coding region of the MC4R gene in 40 morbidly obese subjects from independent families. Here we report a novel heterozygous mutation (N274S) in an adult female obese individual (age: 52 yrs, BMI 41. 7 kg/m^2, height 158 cm, weight: 104 Kg). The sister of the index case (age: 55 yrs, height: 160 cm, weight: 110 Kg, BMI: 43 kg/m^2) also carries the same mutation. Although both sisters were morbidly obese and hypertensive the index case had normal plasma insulin and fasting blood glucose levels whereas her sister had type 2 diabetes mellitus. No abnormalities of the reproductive function were present. Despite marked hyperphagia in childhood both sisters had a history of relatively diminished intensity of appetite after the age of 20. Of notice, index case was diagnosed to have cyclothymia whereas her sister was being treated for bipolar affective disorder. Detailed clinical evaluation revealed normal bone mineral density and serum calcium parameters as well as intact thyroid axis and hypothalamus-pituitary-adrenal axis in both patients. The human MC4-R deficient phenotype resembles the murine deficient state with regard to preserved reproductive function although hyperphagia, increased linear growth and absence of diabetes in mice are not observed in humans. Affected individuals have hyperphagia in childhood, which looses intensity later in life, and they also present with normal height and diabetes mellitus. Accumulating evidence indicate that melanocortin endocrine system or defective melanocortin signaling has inherently different characteristics in mice and humans resembling the variation observed with regard to leptin deficiency in both species.

The melanocortin 4 receptor (MC4-R) is a G-protein coupled receptor that couples through G's to raise intracellular cyclic AMP (cAMP) (1). MC4-R, which is expressed in the hypothalamus, is stimulated by α-MSH (2) and antagonized by agouti-related protein (AGRP) (3). Studies of the MC4-R knockout mice have revealed that mice lacking MC4-R have a predisposition to obesity and hyperinsulinemia first manifested after about 8 weeks of age (4). Recent studies also demonstrated that MC4-R could mediate the control of both metabolic rate and food intake in mice (4,5). Selective blockage of the MC4-R in the brain stimulates food intake in rats (6), and also that MC4 receptor signaling is involved in mediating leptin's inhibitory effect on food consumption (7). Watanobe et al. showed that endogenous MC's may tonically stimulate the surges of various hormones (e.g. LH, PRL) in normally fed rats via the MC4 receptor (8). Thus, it is suggested that MC4-R have a physiological role in regulating reproductive function (8).

Yeo et al. and Vaisse et al. (9,10) reported the first MC4-R mutation in humans in 1998. Later Gu et al. (11) reported two missense mutations in 140 obese subjects investigated and Hinney et al. (12) reported nine missense mutations and two nonsense mutations in 306 obese children. Recently Farooqi et al. (13) reported 6 novel missense mutations while Vaisse et al. (14) reported 8 novel mutations. All affected subjects described have been heterozygous but only one homozygous mutation reported by Farooqi et al. (13).

In our ongoing Turkish Obesity Genome Project, we have previously found a missense leptin gene mutation in a consengenouis Turkish family (15, 16). Here we report a novel MC4-R gene mutation in two members of a Turkish family.

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Subjects and Methods

Patients

Mutational analysis of the MC4-R gene was performed in forty unrelated adult patients (5 males and 45 females). The subjects were recruited from the outpatient clinic of Department of Endocrinology and Metabolism of Gulhane School of Medicine in Turkey. All subjects had a history of severe obesity before the age of 10 and had at least one other obese family member. The study was approved by the local ethical committee of Gulhane Medical School and all subjects gave informed consent for participating in the study.

DNA Study

Genomic DNA was extracted from white blood cells as described previously (17). The coding sequence/region of the MC4-R gene was amplified by the polymerase chain reaction (PCR) using the following primers: forward primer 5'-TGAGACGACTCCCTGACCCAG-3', and reverse primer 5'-CACTGTGAAACTCTGAGCATCC-3'. PCR was performed with a thermal cycler (Perkin Elmer Cetus, USA) under the standard conditions for 30 cycles and 56 0C as annealing temperature. PCR products were separated on regular high melt agarose and sequenced directly by dideoxy chain termination method using the USB 70170 DNA sequencing kit (Amersham, USA).

Metabolic and Endocrine Tests

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Serum samples were analyzed for cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, VLDL cholesterol, glucose, insulin, C peptide, calcium, plasma renin activity, urea, creatinine, free T3, free T4, thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), estradiol, progesterone, testosterone, DHEA-SO4, cortisol, growth hormone (GH), parathormone (PTH), aldosterone by standard assays. Free

urine cortisol was measured in 24-hour urine samples. The percentage of body fat was determined using a bioelectrical impedance device (Bodystat 1500, Bodystat Limited, Douglas, UK). BMD (g/cm2) was determined at the level of the second to fourth lumbar vertebrae (spine) by dual energy X-ray absorptiometry (Norland XR 36-WBL, Fort Atkinson, WI, USA).

Results

We screened the entire coding region of MC4-R gene in 40 morbidly obese adult subjects. A novel heterozygous mutation (N274S) was found in a female (age: 52 yrs, height: 158 cm, weight: 104 kg, BMI: 41. 7 kg/m^2) (Figure 1). The sister of the index case (age: 55 yrs, height: 160 cm, weight: 110 kg, BMI: 43 kg/m^2) was also heterozygous for the same mutation. Both sisters are born to consanguineous parents. Pedigree of the family is shown in Figure 2. Clinical and laboratory features of the sisters carrying the defective gene are given in Table 1.

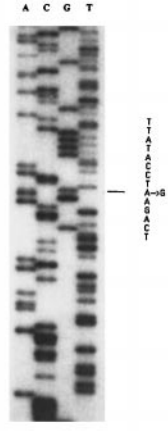

Figure. 1 Sequence analysis for MC4-R gene mutation in codon 274

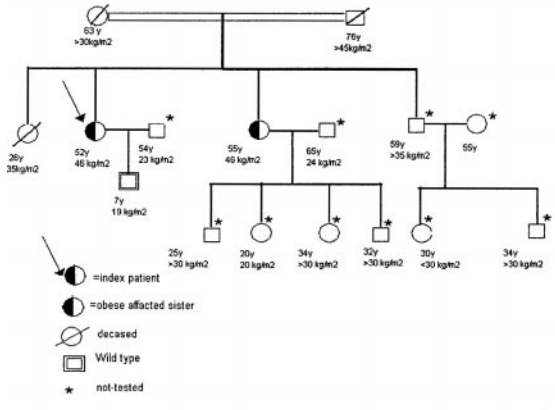

Figure. 2 Genogram of patients with a mutated MC4R gene. Double horizontal bar indicates consanguineous couple.

Both sisters have morbid obesity, increased body fat and normal height (Table1). Sister of the index case has type 2 diabetes mellitus. Index patient had been diagnosed to have cyclothymia and her sister is currently under medication for having bipolar affective disorder. Interestingly, the index case had normal plasma insulin and fasting blood glucose levels and a normal response to oral glucose load (Table 1). Both sisters were hypertensive and had apparently normal reproductive function. Marked hyperphagia was present in childhood in both sisters with subsequent diminished intensity later in life.

Table 1. Clinical features and basal hormone levels in subjects carrying the N274S mutation

	Index Patient	Sister
Clinical data		
Genotype	*Heterozygous*	*Heterozygous*
Gender	*F*	*F*
Age (yr)	*52*	*55*
Height (cm)	*158*	*160*
Weight (kg)	***104***	***110***
BMI (kg/m^2)	***41.*** *7*	*43*
% fat (N:22-30%)	***46. 5***	***47. 1***
BMD of L1-L4 (g/cm^2)	*1,067*	*0,946*
BMC (g)	*57,14*	*46. 18*
t-score	*+0,18*	*-0,92*
Metabolic status		
Urea (5.35-15.7 mmol/L)	*7.85*	*11.42*
Creatinine (38.1-114.4 µmol/L)	*45.7*	*61.0*
Cholesterol (3.8-6.2 mmol/L)	*4.79*	***6.60***
Triglycerides(0.56-2.26 mmol/L)	*2.14*	***3.89***
HDL cholest (0.93-1.68 mmol/L)	***0.98***	***0.77***
LDL cholest. (1.55-4.14 mmol/L)	*109*	*156*
VLDL cholest (0-1.03 mmol/L)	*0.98*	***1.78***
Fasting blood glucose (3.60-5.93 mmol/L)	*4.55*	***13.76***
Calcium (2.12-2.62 mmol/L)	*2.42*	*2.35*
Hormones		
Insulin (<208.3 pmol/L)	*107.6*	*112.5*
C-peptide (0.29-1.32 nmol/L)	*0.69*	*1.65*
Free T3 (0.02-0.06 pmol/L)	*0.055*	*0.058*
Free T4 (10.3-24.4 pmol/L)	*15.4*	*14.1*
TSH (0.4-4.6 mU/L)	*1. 4*	*0. 9*
ACTH (<10.12 pmol/L)	*3.14*	*3.65*
24-h UFC (10-100 µg/24h)	*54*	*ND*
LH (2.5-12.1 IU/L)	***12. 9***	***13. 4***
FSH (1.9-11.6 IU/L)	***17. 1***	***29***
Estradiol (<110.1 pmol/L)	*175.1*	***73.4***
Total testosterone (2.18-4.16 nmol/L)	***0.69***	***1.95***
Prolactin (1.9-25 µg/L)	*14. 1*	*5. 4*
Progesterone (1.24-17.2 nmol/L)	***0.95***	***0. 35***
DHEA-SO4 (0.095-1.16 µmol/L)	***<0.081***	*0.131*
Cortisol (137.9-689.7 nmol/L	*482.82*	*281.41*
Growth hormone (0.06-8.6 µg/L)	*0. 21*	*0. 4*
Parathormone (12-72 ng/L)	*28. 1*	***84. 3***
Aldosterone (0.22-4.76 nmol/L)	*4.38*	*3.24*
Plasma renin activity (0.41-1.38 ng/ml/s)	*0.08*	*1.27*

ND: Not determined, UFC. Urinary free cortisol, Abnormal values are indicated as bold characters

Bone mineral density of the spine, tests of thyroid function and the hypothalamus-pituitary-adrenal axis were normal in both sisters. Cortisol and ACTH diurnal rhythm were also normal (data not shown). Growth hormone, PRL, aldosterone and renin activity were in the normal range. Parathormone level is normal in the

index case but it is slightly higher in the sister. DHEAS levels were decreased in both patients which is a consistent finding among obese subjects. Although there was no evidence of a history of disturbed reproductive function, FSH and LH levels were elevated associated with a decrease in testosterone, estradiol and progesterone levels indicating unovulatory cycles in the index case and gonadotropin levels were also high in the sister representing a surgical menopausal state.

Discussion

We have identified a novel heterozygous missense mutation (N274S) in the MC4-R gene associated with morbid obesity and psychiatric disorders in two female siblings from a Turkish family.

Hyperphagia and early onset obesity during the childhood was prominent similar to previously described patients carrying a mutated MC4-R gene (13).

Of notice, basal insulin and fasting blood glucose levels were normal in the index case whereas the sister had overt type 2 diabetes mellitus. The prevalence of type 2 diabetes and impaired glucose tolerance among humans with a defective MC4-R gene are very similar to those in obese subjects (14). Farooqi et al. reported normal fasting plasma glucose levels despite hyperinsulinemia in a group of patients with a MC4-R mutation (13). The MC4-R knockout mouse also displays hyperinsulinemia, but not diabetes (4).

Detailed evaluation of various endocrine systems such as the thyroid and adrenal axis revealed no abnormal function. Moreover, GH, PRL and renin aldosterone system were also normal. Decreased DHEAS levels presumably represents a common feature of morbid obesity (18).

One may also argue that slightly elevated parathormone levels may be a feature of some morbid obese patients (19, 20). No abnormalities of pubertal development and reproductive function were present as previously reported in other subjects carrying the mutation. Although previous studies have demonstrated that other monogenic forms of human obesity were associated with several endocrine abnormalities (15,16,21,22) obese humans with mutations in the MC4-R gene seems to have no specific endocrine abnormalities (9-14).

However, Farooqi et al. have reported that two of four nonobese heterozygous adults were significantly hypertensive and even children carrying the mutation may have elevated blood pressures (13). Yeo et al. (9) and Farooqi et al. (13) reported tall stature and an increased growth velocity during childhood in the majority of affected subjects. Although both sisters were hypertensive their heights were normal.

BMD and BMC were in normal range in our patients, although Farooqi et al. reported significantly increased BMC and BMD in their mutated patients (13). The obese are usually protected against osteoporosis and have increased bone mineral density and plasma leptin levels (23, 24). The mechanism by which obesity protects people from osteoporosis is still a mystery, although a recent study suggested a central control of bone mass by leptin (25). Thus, we consider that MC4R mutation has no particular effect on bone mass.

Psychiatric disorders have not been reported previously in humans with MC4-R mutation although it is not clear whether this is an associated feature or a coincidence. A genome-wide survey has been suggested a region between D13S71 and D13S274 on 13q32 to be linked to bipolar disorder (26). Thus, further studies are needed to identify an association between MC4-R and bipolar disorders.

Our data demonstrates that heterozygous mutation in MC4-R predisposes to nonsyndromic obesity in humans as observed in previous studies. The lack of human MC4-R results in most of the features resembling murine knockout model with regard to preserved reproductive function; however, hyperphagia, increased linear growth and absence of diabetes in mice does not seem to be similarly penetrated in humans. Such phenotypic variations in mice and humans are also noteworthy in the context of data observed from leptin deficient humans (16). This type of variability might be due to either differential regulation of the melanocortin endocrine system or inherently different characteristics of defective melanocortin signaling in mice and humans. Thus, observations on melanocortin deficiency in mice may not be largely applicable for humans.

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