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PAPER

A novel nonsense mutation in the melanocortin-4 receptor associated with obesity in a Spanish population

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BACKGROUND: In recent years, several groups have reported dominant inheritance of obesity conferred by missense, nonsense and frameshift mutations in the melanocortin 4 receptor gene (*MC4R*). Hence, *MC4R* is involved in the most common monogenic form of human obesity described so far.
OBJECTIVES: In this context, we screened a Spanish population, composed of obese subjects and normal weight controls, for mutations in the *MC4-R* by single-strand conformational polymorphism (SSCP).
SUBJECTS AND METHODS: Overall 313 individuals, 159 obese subjects (body mass index: BMI: 37.6 kg/m^2, 95% CI: 36.7–38.5 kg/m^2) and 154 normal weight control subjects (BMI: 22.3 kg/m^2, 95% CI: 22.0–22.6 kg/m^2) were screened for *MC4-R* mutations.
RESULTS: We detected a novel nonsense mutation at codon 16 of the *MC4-R* in an obese female (BMI: 30.0 kg/m^2) and a previously described missense mutation (Val-253-Ile) located within the sixth trans-membrane domain of the *MC4-R* in a normal weight individual (BMI: 19.0 kg/m^2). The polymorphism Val-103-Ile was detected in one obese individual, while four subjects (two cases and two controls) with the polymorphism Ile-251-Leu were found.
CONCLUSIONS: We have identified a novel nonsense mutation (Trp-16-Stop) that, based on previously described frameshift and nonsense mutations, most likely results in dominantly inherited obesity. Within this Spanish population, the frequency of the Ile-251-Leu polymorphism of the *MC4R* was similar in obese and control subjects (about 1.3%), while the polymorphism Val-103-Ile was only detected in an obese individual (0.6%).
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Keywords: melanocortin 4 receptor; SSCP; polymorphism

Introduction

Dominant inheritance of obesity conferred by missense, nonsense and frameshift mutations in the melanocortin 4 receptor gene (*MC4R*) has been extensively reported in many populations including French, English, German, American and Italian individuals.[1–12] Several mutations of the *MC4R*, mainly one nonsense (Tyr-35-Stop) and three frameshift mutations (732-733insGATT, 631-634delCTCT, 47-48insG) can be considered loss-of-function mutations and are associated with dominant inheritance of obesity. Functional studies showed that many of the missense muations also lead to a loss of function of the MC4R.[5,6,8,13] However, other mutations (ie Thr-11-Ser, Arg-18-Cys) and two polymorphisms (Val-103-Ile, Ile-251-Leu) did not modify the function of the MC4R *in vitro*.[5,7] It has been estimated that 2–4% of extremely obese individuals harbor functionally relevant *MC4R* mutations.[14] In this Spanish study, we assessed mutation rates in 159 obese cases and 154 normal weight controls. The repeated comparison of mutation rates between obese and control study groups is useful for assessing the functional implication of *MC4R* mutations in the etiology of obesity.

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Materials and methods

The study population comprised 313 Spanish subjects, aged 20–60 y and it is based on a case–control design. Cases

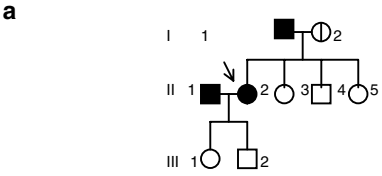
(BMI>30 kg/m^2) were recruited at the Endocrinology Department at the Navarra Hospital and controls (BMI < 25 kg/m^2) were ascertained by the Occupational Health Department at the same hospital, between January 1999 and June 2000. Exclusion criteria were current hormonal treatment or obesity secondary to endocrine disorder or serious intercurrent illness. Subjects with type II diabetes, not receiving glucose-lowering agents, were eligible as cases (9%). In total, 159 obese patients (BMI mean: 37.6 kg/m^2; 95% CI: 36.7–38.5 kg/m^2) and 154 normal weight subjects (BMI mean: 22.3 kg/m^2; 95% CI: 22.0–22.6 kg/m^2) were selected. Controls were healthy subjects having a BMI < 25 kg/m^2 with no apparent disease and blood pressure below 120/90. Responses rates were acceptable (65% for cases and 75% for controls) and the interviews were all conducted in a medical enviroment. The study was approved by the Ethics Committee of the University of Navarra and all subjects provided written informed consent for participation. The reported investigation has been carried out according to the principles of the Declaration of Helsinki II.

Anthropometric measurements were made by standard procedures and fat mass was measured by bioelectrical impedance. Following a 12 h fast, venous blood samples were obtained and serum glucose and lipids were measured by enzymatic methods. Serum insulin was measured by radioimmunoassay (TK/N1, Diagnostic Product Corporation, Los Angeles-California, USA) and plasma leptin by enzyme-immune assay (EIA-1863, DRG Diagnostics, Germany) as described elsewhere.[15] Blood samples were taken for the extraction and characterization of genomic DNA from leukocytes. Single strand Conformational polymorphism (SSCP) analysis for mutations in the *MC4-R* was performed as previously reported.[3] By sequencing of PCR products showing an aberrant SSCP pattern, we identified several heterozygous carriers of mutations and polymorphisms (Figure 1b). The Trp-16-Stop mutation was additionally verified by digestion of the *MC4R* PCR-fragment 1;[3] whereby the 16-Trp allele was digested with *Bsp*LI (Fermentas) and the 16-Stop allele remained uncut.

Results

We identified four different variants: a novel nonsense (Trp-16-Stop) mutation, a previously described missense mutation (Val-253-Ile), as well as two known polymorphisms Val-103-Ile and Ile-251-Leu (Table 1). The clinical characteristics of the *MC4R* mutation carriers are indicated in Table 2. The Val-103-Ile polymorphism was detected in one (BMI: 35.4 kg/m^2) out of the 159 obese individuals, while we identified two controls and two obese subjects with the Ile-251-Leu polymorphism.

The obese female heterozygous for the Trp-16-Stop mutation (II2 in Figure 1a) reported early onset of obesity at age 8. She additionally reported having continuously restricted her energy intake in the sense of being on a constant diet. Her maximum weight was 100 kg (BMI 34.6 kg/m^2, at age 30 y).



Phenotypic data

	I1	I2	II1	II2	II3	II4	II5	III1	III2
Age in years	58	53	37	32	34	29	24	13	9
BMI in kg/m^2	30	28	30	30	22	23	22	24	15

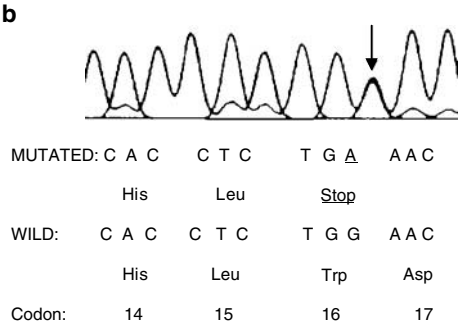


Figure 1 (a) Pedigree of the obese index patient harboring the Trp-16-Stop mutation in the *MC4R*. (b) *MC4R* partial sequencing and deduced amino-acid sequences of the patient II-2. The arrow indicates the position of the G/A substitution. The base substituted is underlined. Grey symbols correspond to obese subjects, a middle line represents carriers of the Trp-16-Stop of the *MC4R*, an arrow indicates the index patient.

Recently, an abdominal lipectomy was performed for cosmetic reasons. She inherited the mutation from her mother (I2 in Figure 1a). Her mother reported adolescenct onset of obesity. She has also been continuously attempting to restrict her energy intake, her maximum weight was 80 kg at age 30 y (max BMI: 30.5 kg/m).

Hormone measurements were performed in the index proband. The corticotropic and thyrotropic axe was normal (ACTH = 8.5 ng/l [0.1–46]; cortisol 08.00 h 20 μg/dl [5–25]; cortisol after dexamethasone (Nugent test): 0.6 μg/dl; T4 free = 1.1 ng/dl; TSH = 0.72 mIU/l [0.35–4.0]). The mother of the index proband, who also carries the nonsense mutation, was not avalilable for this determination.

Discussion

Here we report for the first time the nonsense mutation Trp-16-Stop in the *MC4R*. The female index patient is not characterized by any readily detectable phenotypic abnormality other than early onset obesity. She inherited the mutation from her mother, who is also a heterozyous carrier and who has a history of obesity. This novel nonsense

Table 1 *MC4R* mutation screening in obese and normal weight individuals

Base change	*Effect on amino-acid sequence*	*Control* (n=*154*)	*Obese* (n=*159*)
G-48-A	Trp-16-Stop[a]	0	1
G-757-A	Val-253-Ile	1	0
A-307-G	Val-103-Ile	0	1
A-751-C	Ile-251-Leu	2	2

Both groups were screened by SSCP.
[a]Novel mutation in the *MCR-4* sequence.

Table 2 Phenotypic characteristics of *MC4R* mutation carriers in comparison with the complete screened study groups

Variable	*Control*	*Obese*	*W16X*	*V253I*	*V103I*	*I251L*		*I251L*	
	(n=*154*)	(n=*159*)	*Obese*	*Control*	*Obese*	*Control 1*	*Control 2*	*Obese 1*	*Obese 2*
Age (y)	39 [37–40]	42 [41–44]	32	26	39	42	47	48	30
Sex (F/M)	113/41	139/20	F	F	F	F	F	F	F
Current weight	61 [59–63]	96 [93–98]	85	55	80	52	51	93	77
BMI (kg/m^2)	22.3 [22.0–22.4]	37.6 [36.7–38.5]	30.0	19.0	35.4	20.8	24.3	39.9	30.1
WHR	0.80 [0.79–0.80]	0.89 [0.88–0.90]	0.96	0.84	0.92	0.74	0.75	0.92	0.83
Max weight (kg)	65 [64–67]	99 [97–102]	94	58	80	55	56	93	82
% fat mass	28 [27–28]	43 [42–45]	43	27	41	26	32	39	38
SBP (mmHg)	111 [109–113]	138 [135–141]	125	100	140	110	100	140	120
DBP (mmHg)	69 [68–70]	88 [86–90]	85	60	90	60	70	90	80
Glucose(mM/l)	5.1 [5.0–5.3]	5.6 [5.4–5.9]	6.3	4.8	4.6	5.8	4.5	5.5	4.8
Insulin (pM/l)	54 [49–59]	149 [126–171]	480	54	66	67	35	132	138
TC (mM/l)	5.1 [4.9–5.2]	5.4 [5.2–5.5]	6.8	6.0	4.5	4.8	5.3	4.3	5.1
TG (mM/l)	0.7 [0.7–0.8]	1.3 [1.2–1.4]	3.1	0.6	1.4	0.6	0.7	1.1	1.4
Leptin (ng/ml)	7.9 [6.7–9.1]	34.4 [29.5–39.3]	28.2	4.7	27.0	5.4	5.8	34.8	18.4

Values are mean [95% CI] when indicated. Max: maximum; BPS, BPD: systolic and diastolic blood pressure; TC: total cholesterol; TG: triglycerides.

mutation at codon 16 of the *MC4R* should lead to a receptor that is truncated at the N-terminal extracellular domain. It resembles the nonsense mutation Tyr-35-Stop, which was also associated with obesity.[3] It is readily evident that the gene products of these two nonsense mutations cannot function based on previous pharmacological characterizations of other nonsense and frameshift mutations.[1,4]

In a normal weight control subject, we detected a previously described missense mutation (Val-253-Ile) that is located within the sixth trans-membrane domain of the MC4R.[4,7] Presently, few missense variants of *MC4R* have been described in normal weight individuals.[4,5]

The Ile-251-Leu polymorphism was detected in two normal weight and two obese subjects, a similar result was reported by Vaisse *et al*[7] and Hinney *et al*[3] in a French and a German study, respectively. However, Farooqi *et al*[4] found more carriers of this polymorphism among obese individuals. Functional analyses revealed that this missense variant is indistinguishable from the wild-type receptor,[7] so that this variant presumably represents a polymorphism, which is not functionally relevant. The polymorphism Val-103-Ile has been reported in many individuals of different ethnic origins with frequencies ranging from 0.5 (8) to 5.1 (3) percent. In a British study group, Gotoda *et al*[16] described similar allele frequencies in obese and normal weight subjects. Most of the reports did not find association with obesity.[3–5,7,16]. This corresponds with normal signaling properties of the 103-Ile receptor variant in functional studies.[5,13] In our study, we only found one obese subject carrier of the Val-103-Ile polymorphism.

In conclusion: (a) a novel nonsense mutation in the *MC4R,* most likely underlying dominantly inherited obesity, was identified in an obese female; (b) within the Spanish population, the frequency of the Ile-251-Leu polymorphism of the *MC4R* was similar in obese and normal weight subjects (about 1.3%), while the polymorphism Val-103-Ile was only detected in an obese individual (0.6%).

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References

1 Vaisse C, Clement K, Guy-Grand B, Froguel P. A frameshift mutation in human MC4R is associated with a dominant form of obesity. *Nat Genet* 1998; **20**: 113–114.
2 Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, O'Rahilly S. A frameshift mutation in MC4R associated with dominantly inherited human obesity. *Nat Genet* 1998; **20**: 111–112.
3 Hinney A, Schmidt A, Nottebom K, Heibult O, Becker I, Ziegler A, Gerber G, Sina M, Gorg T, Mayer H, Siegfried W, Fichter M, Remschmidt H, Hebebrand J. Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. *J Clin Endocrinol Metab* 1999; **84**: 1483–1486.
4 Farooqi IS, Yeo GS, Keogh JM, Aminian S, Jebb SA, Butler G, Cheetham T, O'Rahilly S. Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. *J Clin Invest* 2000; **106**: 271–279.
5 Gu W, Tu Z, Kleyn PW, Kissebah A, Duprat L, Lee J, Chin W, Maruti S, Deng N, Fisher SL, Franco LS, Burn P, Yagaloff KA, Nathan J, Heymsfield S, Albu J, Pi-Sunyer FX, Allison DB. Identification and functional analysis of novel human melanocortin-4 receptor variants. *Diabetes* 1999; **48**: 635–639.
6 Sina M, Hinney A, Ziegler A, Neupert T, Mayer H, Siegfried W, Blum WF, Remschmidt H, Hebebrand J. Phenotypes in three pedigrees with autosomal dominant obesity caused by haploinsufficiency mutations in the melanocortin-4 receptor gene. *Am J Hum Genet* 1999; **65**: 1501–1507.
7 Vaisse C, Clement K, Durand E, Hercberg S, Guy-Grand B, Froguel P. Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. *J Clin Invest* 2000; **106**: 253–262.
8 Miraglia Del Giudice E, Cirillo G, Nigro V, Santoro N, D'Urso L, Raimondo P, Cozzolino D, Scafato D, Perrone L. Low frequency of melanocortin-4 receptor (MC4R) mutations in a Mediterranean population with early-onset obesity. *Int J Obes Relat Metab Disord* 2002; **26**: 647–651.
9 Hebebrand J, Sommerlad C, Geller F, Gorg T, Hinney A. The genetics of obesity: practical implications. *Int J Obes Relat Metab Disord* 2001; **25**(Suppl 1): S10–S18.
10 Kobayashi H, Ogawa Y, Shintani M, Ebihara K, Shimodahira M, Iwakura T, Hino M, Ishihara T, Ikekubo K, Kurahachi H, Nakao K. A Novel homozygous missense mutation of melanocortin-4 receptor (MC4R) in a Japanese woman with severe obesity. *Diabetes* 2002; **51**: 243–246.
11 Rosmond R, Chagnon M, Bouchard C, Bjorntorp P. A missense mutation in the human melanocortin-4 receptor gene in relation to abdominal obesity and salivary cortisol. *Diabetologia* 2001; **44**: 1335–1338.
12 Mergen M, Mergen H, Ozata M, Oner R, Oner C. A novel melanocortin 4 receptor (MC4R) gene mutation associated with morbid obesity. *J Clin Endocrinol Metab* 2001; **86**: 3448.
13 Ho G, MacKenzie RG. Functional characterization of mutations in melanocortin-4 receptor associated with human obesity. *J Biol Chem* 1999; **274**: 35816–35822.
14 Cone RD. Haploinsufficiency of the melanocortin-4 receptor: part of a thrifty genotype? *J Clin Invest* 2000; **106**: 185–187.
15 Corbalan MS, Marti A, Forga L, Martínez-Gonzalez MA, and Martínez JA. The risk of obesity and Trp64Arg polymorphism of the β_3-adrenergic receptor: effect modification by age. *Ann Nutr Metab* 2002; **46**: 152–158.
16 Gotoda T, Scott J, Aitman TJ. Molecular screening of the human melanocortin-4 receptor gene: identification of a missense variant showing no association with obesity, plasma glucose, or insulin. *Diabetologia* 1997; **40**: 976–979.