

Mutational Analysis of the Pro-opiomelanocortin Gene in French Obese Children Led to the Identification of a Novel Deleterious Heterozygous Mutation Located in the α -Melanocyte Stimulating Hormone Domain

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ABSTRACT: The pro-opiomelanocortin (POMC) plays a key role in body weight regulation, where its derived peptides mediate leptin action *via* the hypothalamic melanocortin 4 receptor (MC4R). The pathogenic effects of POMC mutations have been challenged in obesity. Our aim was to assess the relevance of POMC mutations in a cohort of French obese and nonobese children. Direct sequencing of the POMC gene was performed in 322 obese and 363 control unrelated children. Functional studies for the novel Phe144Leu mutation included the response to α -melanocyte stimulating hormone (α MSH) and a competitive binding assay. POMC mutations were identified in 3.72% of obese [95% confidence interval (CI): 1.66–5.80] and 2.20% of control (95% CI: 0.69–3.71) subjects. The novel mutation located in the α MSH region of the POMC gene (Phe144Leu) was found in one obese child and was transmitted by the obese father. Functional studies showed that MC4R activation in response to Leu144 α MSH was almost completely abolished due to a dramatically altered binding of Leu144 α MSH to MC4R. The frequency of POMC mutations is not significantly different between obese and control children in our cohort. The novel heterozygous mutation Phe144Leu leading to the absence of melanocortin signaling was associated with early-onset obesity suggesting its pathogenic role. (*Pediatr Res* 63: 211–216, 2008)

The proopiomelanocortin (POMC) is a complex propeptide encoding a number of melanocortin peptides that are released by tissue-specific proteolytic processing. These pep-

tides have important roles in a range of functions such as skin pigmentation, control of adrenal function, and body weight regulation *via* the leptin/melanocortin pathway (1). The production of POMC in the central nervous system is stimulated by leptin and its posttranslational process leads to the generation of different peptides (2). The nature of POMC-derived peptides depends on the type of endoproteolytic enzymes present in specific brain areas. In the anterior pituitary, the presence of the proconvertase (PC) 1 enzyme allows the production of adrenocorticotrophic hormone (ACTH) and β -lipotropin peptides, while the simultaneous presence of PC1 and PC2 in the hypothalamus determines the production of α -, β -, γ -melanocyte-stimulating hormone (α -, β -, γ MSH) and β -endorphins. POMC knockout mice become obese due to the loss of the anorexigenic action of α MSH on melanocortin-4 receptor (MC4R) expressing neurons. They have no adrenal function because of the lack of ACTH production and present variable alterations in coat pigmentation due to the absence of α MSH in the skin (3). The phenotype of POMC-deficient mice resembles that of patients with rare mutations that completely prevent POMC-derived peptide production. To date, six families with children carrying homozygous or compound heterozygous loss-of-function mutations in POMC have been described. The children in these families display a hypocortisolism, early-onset obesity, and variable alterations of skin and hair pigmentation, thereby defining a new rare syndrome of obesity associated with POMC deficiency (4,5).

The POMC gene is located in the human chromosome 2, which is a region strongly linked with leptin levels and to a

Received May 15, 2007; accepted September 26, 2007.

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Programme Hospitalier de Recherche Clinique Direction de la Recherche Clinique/Assistance Publique-Hopitaux de Paris (contract grant number PHRC AOM 96088), BQR of Paris 6 University. Funding was obtained by the program GIP-ANR (No. 06-CPER-194-02). B.D. was supported by a grant from the AFERO (Association Française d'Etudes et de Recherches sur l'Obésité) and by a "Bourse Collery de l'Académie Nationale de Médecine." C.L.B. was supported by a fellowship from the Danone Institute (Prix Alimentation et Santé 2004). B.E. was supported by UCSF Endocrinology and Metabolism Training Grant. The work of B.E. and C.V. was supported by NIH DK068152-01.

B.D. and C.L.-B. equally contributed to this work.

Abbreviations: ACTH, corticotropin; MC4R, melanocortin 4 receptor; MSH, melanocyte-stimulating hormone; POMC, pro-opiomelanocortin; PC, proconvertase

lesser extent with obesity (6–9). It was considered a strong positional candidate, and direct gene screening revealed several polymorphisms, generally with low frequency, located in both coding and noncoding regions (10). Variants of the POMC gene were found in German (11,12), Danish (13), Swedish (14), Italian (15,16), and English (17,18) obese children and young adults and in French diabetic and obese adults (19). These variations included base insertion or deletion, missense, or silent mutations and were generally uncommon. Most of these variants were not associated with obesity-related phenotypes and generally had no functional consequences, except for mutations involving β MSH domain (Arg236Gly and Tyr221Cys) (12,17,18). The frequency of the Arg236Gly mutation was shown to be mildly increased in U.K. obese children, and functional analyses revealed that this mutation prevents the normal processing of β MSH and β -endorphin, resulting in a fusion protein (17). Able to bind MC4R, this aberrant peptide leads to a decreased activation of the receptor. Recently, the mutation Tyr221Cys, also located in the β MSH domain of POMC, was implicated in early-onset obesity with deleterious *in vitro* functional effects (12,18). Those findings suggested that β MSH might play a critical role in weight regulation in humans. Thus, further investigations of the POMC gene in obese and nonobese populations are needed to determine the contributing role of POMC gene variants in human obesity.

In the present study, we report the mutational analysis of the POMC gene in a French cohort of 322 obese children and 363 controls. We also describe a novel heterozygous mutation in the α MSH domain of the POMC gene associated with early-onset obesity and with deleterious *in vitro* functional effects.

MATERIALS AND METHODS

Direct POMC gene sequencing. This study was approved by the local ethics committee, and informed consent was obtained from all participating subjects. The genomic DNA of all subjects was isolated from 10 mL of venous whole blood (Wizard Genomic DNA Purification Kit, Promega Madison, WI). Direct sequencing (Sequencer Applied Biosystem) of the coding region of the POMC gene was performed in 322 unrelated obese children and in 363 nonobese children. Obese children [mean age, 12.8 y \pm 2.8 standard deviations (SDs); mean body mass index (BMI) Z score = 4.3 \pm 1.2 SDs; BMI Z score range, 2.0–10.2 SDs] were prospectively recruited between 2001 and 2004 in three different departments of pediatric nutrition located in Paris, Caen, and Margency, France [as described in Dubern *et al.* (20)]. In the present study, obesity was defined as a BMI Z score at least 2 SDs above the mean age and sex-specific BMI values in children in France (21). The control group included 363 nonobese children (mean age, 11.5 y \pm 0.6 SD; mean BMI Z score = 0.2 \pm 1.0 SD; BMI Z score range, -2.96 to 1.93 SDs). These children were part of a randomized, controlled ongoing field trial (ICAPS) in middle school's first-level adolescents from eight randomly selected schools in the department of the Bas-Rhin (Eastern France) (22).

Genotyping of the Phe144Leu POMC mutation in the proband's family was also performed by direct sequencing (Sequencer Applied Biosystem) of the coding regions of the POMC gene. Primers and conditions can be provided upon request. Nucleotides and amino acids were numbered according to GenBank accession numbers V01510 and NP 000930, respectively.

Clinical and biochemical data. All obese patients underwent a physical examination with anthropometric measurements and Tanner stage evaluation by the same investigator in each center. Height and weight curves were obtained from the medical health records of the subjects.

In the patient carrying the Phe144Leu mutation, body composition (fat-free mass and body fat) was measured using whole-body dual-energy x-ray absorptiometry (Hologic QDR 2000 unit, Waltham, MA). The percentage body fat was calculated as the ratio of total body fat mass over total body mass. Blood samples were collected in the morning after an overnight fast.

Total cholesterol and triacylglycerol were measured using an enzymatic assay (Boehringer, Mannheim, Germany). Plasma leptin concentrations were measured by radioimmunoassay (RIA) (LINCO Research, Inc., St. Louis, MO). Oral glucose tolerance test was performed by giving 1.75 g glucose/kg body weight (maximum, 75 g). Plasma glucose and insulin concentrations were measured in the fasting state and 30 and 120 min after glucose ingestion using the glucose oxidase method and a RIA with polyclonal antibodies (INSI-PR, CIS Bio International, Gif-sur-Yvette, France), respectively. Hypothalamic-pituitary-adrenal axis exploration included urinary free cortisol and a dynamic test of adrenal axis with measurements of serum cortisol and ACTH by standard immunoassays. To explore thyroid axis, serum free thyroxine (T_4) and thyroid-stimulating hormone (TSH) were measured by standard immunoassays. Basal resting metabolic rate was performed using indirect calorimetry (Deltatrac II monitor, Datex Instrumentarium Corp., Helsinki, Finland).

Human MC4R (hMC4R) activation by the α MSH product of Phe144Leu POMC. Leu144 α MSH (Leu at position 7 of α MSH) and Phe144 α MSH (Phe at position 7) were synthesized and purified by high-performance liquid chromatography (Genemed Synthesis Inc., South San Francisco, CA). MC4R activity was measured by analyzing its ability to activate expression of a cyclic adenosine monophosphate (cAMP)-inducible luciferase gene in HEK 293 (23). HEK 293 cells stably expressing a cAMP-inducible luciferase gene were transiently transfected with the hMC4R and stimulated for 6 h with medium alone, increasing amounts of Phe144 α MSH or Leu144 α MSH, after which luciferase activity was measured as previously described (23). Data points represent means of five determinations and error bars indicate SDs.

Competitive binding assay. HEK 293 cells stably transfected with the hMC4R were incubated with [¹²⁵I]NDP α MSH in the presence of increasing concentrations of Phe144 α MSH or Leu144 α MSH. Results are expressed as a percentage of total specific binding (B_{max}). The curve is fitted using nonlinear regression analysis and one-site competition model (GraphPad Prism, graph software, San Diego, CA). Each point is the mean of triplicate values. Error bars indicate SDs.

RESULTS

Frequency of POMC mutations in obese and lean French children and identification of a patient carrying the Phe144Leu POMC mutation.

By screening 322 severely obese children for POMC mutations, nine mutations were identified (Asp53Gly, Gly96Cys, Pro132Ala, Arg236Gly, Glu214Gly, Ala195Thr, Leu209Pro, Phe87Leu, Phe144Leu) in 12 unrelated individuals (3.72%, 95% CI: 1.66–5.80). Among them, six have not been previously reported (Asp53Gly, Phe87Leu, Gly96Cys, Pro132Ala, Phe144Leu, Leu209Pro). In the control group, two novel mutations (Asn91Ser, Glu57Lys) were uniquely detected, whereas two mutations (Glu214Gly, Ala195Thr) were found in both obese and control populations (Table 1 and Fig. 1). Four obese patients and one lean control were carriers of the Asp53Gly mutation (1.24%). Overall, the frequency of POMC gene variations was not significantly different between obese (3.72%, 95% CI: 1.66–5.80) and controls [2.20%, 95% CI: 0.69–3.71, *p* = not significant (NS)] in this group of French children. Clinical and biological characteristics of POMC mutation carriers (obese and controls) are shown in Table 2. No difference in weight and BMI Z score was observed between POMC genetic variant carriers and noncarriers in the obese and lean groups, respectively (data not shown).

Because most of the detected mutations occurred in regions of the POMC propeptide with uncertain biological functions, we concentrated our study on the novel heterozygous missense mutation Phe144Leu located in the α MSH coding region of POMC gene. The Phe144Leu mutation was detected in only one obese child and was absent in the control population. Segregation analysis of the mutation in the family showed that the mutation was present in the obese father (Fig. 2A). The

Table 1. Frequency of POMC gene variations in obese and control children

Nucleotide change	Amino acid change	Obese (n = 322)	Controls (n = 373)	χ^2
158 a>g	Asp53Gly	4 (1.24%)	1 (0.27%)	NS
287 c>t	Gly96Cys	1 (0.31%)	0	
395 c>g	Pro132Ala	1 (0.31%)	0	
707 c>g	Arg236Gly	1 (0.31%)	0	
642 a>g	Glu214Gly	1 (0.31%)	4 (1.07%)	
584 g>a	Ala195Thr	1 (0.31%)	1 (0.27%)	
627 t>c	Leu209Pro	1 (0.31%)	0	
262 c>a	Phe87Leu	1 (0.31%)	0	
273 a>g	Asn91Ser	0	1 (0.27%)	
169 g>a	Glu57Lys	0	1 (0.27%)	
431 t>c	Phe144Leu	1 (0.31%)	0	
POMC gene variations frequency		3.72%, 95% CI: 1.66–5.80	2.20%, 95% CI: 0.69–3.71	NS

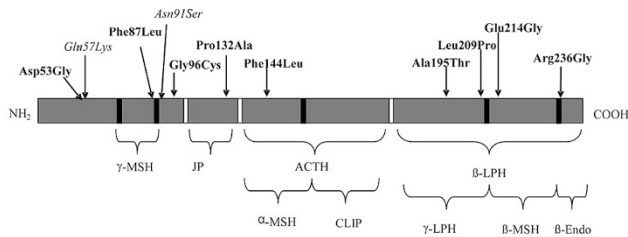


Figure 1. POMC protein and location of the variants in obese and lean children. White and black boxes are for the cut sites for PCs. Italics: mutations identified only in controls. JP, joint peptide; CLIP, corticotropin-like intermediate peptide; LPH, lipoprotein hormone; Endo, endorphin.

other available family members were not carriers of the mutation and were neither overweight nor obese.

Clinical and biologic phenotype of the phe144leu mutation carrier. The Phe144Leu mutation carrier was a 12-y-old obese girl from a nonconsanguineous family. She was born at term and had a normal birth weight (3000 g). She had no red hair and no clinical evidence of adrenal impairment. At age 12 y, the patient's BMI Z score was 2.7 SDs with normal puberty (Tanner stage 4). Her fat mass was 31.45% (18.7 kg) of her total body mass. The BMI curve showed a preserved but early (at age 2 y) adiposity rebound in the absence of any dietary intervention (Fig. 2B). The age at obesity onset, defined as age with BMI above the 97th percentile, was 4.5 y. We compared the BMI evolution of the proband with that of subjects lacking leptin receptor (LEPR) signaling and with that of subjects heterozygous for MC4R mutations (24) (Fig. 2B). In contrast to LEPR-deficient patients, the BMI curve of the proband did not show a dramatic augmentation. When compared with young MC4R mutation carriers (20, unpublished data), the BMI curves were close during the first 4 y of life between MC4R mutation carriers and the girl carrying the Phe144Leu mutation. Differences in BMI evolution markedly appeared after the age of 5 y because the BMI of the POMC mutation carrier showed only minor aggravation compared with MC4R subjects displaying a rapid increase in BMI in the absence of any dietary intervention.

Endocrine and metabolic explorations in the proband showed only a moderate hyperinsulinemia with a plasma insulin level of 16.9 μ UI/mL 120 min after glucose ingestion with normal glucose levels (Table 3). Hypothalamic-pituitary-

adrenal axis explorations were normal (urinary free cortisol, dynamic test of adrenal axis), as well as thyroid axis explorations (free T₄ and TSH) (Table 3). Circulating leptin measurement (leptin 20 ng/mL) was in agreement with her corpulence (leptin/kg fat mass, 1.1). Lipid metabolism was unremarkable (total cholesterol, 1.7 g/L; triacylglycerol, 0.9 mM). At age 12 y, the resting metabolic rate evaluated by indirect calorimetry was normal for her body composition (1260 kcal /24 h, 39 kg fat-free mass).

Functional effect of the Phe144leu POMC mutation. We evaluated the ability of Leu144 α MSH (mutated POMC allele) to activate hMC4R by comparing both cAMP production and the binding to the receptor with that of Phe144 α MSH (wild-type allele). In our assay, hMC4R activation in response to Leu144 α MSH was almost completely abolished (EC₅₀ not determinable) compared with that with Phe144 α MSH (EC₅₀ \pm SD = 1.5 \pm 0.7 nM) (Fig. 3A). This result was due, at least partially, to the dramatically altered binding of Leu144 α MSH to hMC4R (Fig. 3B).

DISCUSSION

We report here the screening of the POMC gene in a cohort of obese and nonobese French children. A novel mutation (Phe144Leu) located in the α MSH domain of the POMC gene associated with early-onset obesity was detected. *In vitro* studies revealed that the Phe144Leu amino acid substitution resulted in a deleterious functional effect with a complete lack of hMC4R activation due to a dramatic impairment of mutant α MSH binding. The Phe144Leu mutation was found in a 12-y-old girl with early-onset obesity. No other associated phenotypes such as adrenal insufficiency and hypopigmentation were noted in contrast to the phenotype in patients with POMC-null homozygous mutation (4). This may be explained by the complex regulation of pigmentation characterized by high interindividual variability. More specifically, pigmentation in humans varies greatly between individuals and particularly among ethnic groups, primarily because of several differences in melanin (*e.g.* size, shape, density, and type) and the multiple polymorphisms in melanocortin 1 receptor that are capable of modulating its basal activity (10). Several observations, including this one, suggest that the skin and hair phenotype might vary according to the ethnic origin of POMC

Table 2. Characteristics of POMC mutations carriers

Subjects	POMC mutation	Age (y)	Sex	BMI Z (SD)	Age at obesity onset (y)	Ins T0 ($\mu\text{U/mL}$)	Gly T0 (mmol/L)	HOMA	TG (mmol/L)	Chol (g/L)	Leptin (ng/mL)
Obese											
1	Asp53Gly	5	F	4.7	5	4.1	4.3	0.8	0.5	0.7	ND
2	Asp53Gly	7	M	6.7	0.5	13.0	5.1	3.0	0.6	1.7	17.5
3	Asp53Gly	8	M	5.2	4	14.0	4.8	3.0	0.5	1.5	16.0
4	Asp53Gly	10	F	4.7	6	19.8	4.8	4.2	1.3	2.1	26.0
5	Gly96Cys	13	F	3.6	?	14.0	4.9	3.0	0.8	1.4	21.0
6	Pro132Ala	17	F	3.8	?	16.9	5.0	3.7	0.9	1.5	ND
7	Arg236Gly	15	F	1.7	5	ND	ND	ND	ND	ND	ND
8	Glu214Gly	11	F	3.3	2	26.0	4.9	5.7	0.8	0.9	12.0
9	Ala195Thr	12	F	5.8	?	ND	ND	ND	ND	ND	ND
10	Leu209Pro	14	M	3.9	3.2	18.0	5.1	4.1	0.8	1.5	19.4
11	Phe87Leu	7	F	6.2	?	15.0	5.0	3.3	0.6	1.8	21.0
12	Phe144Leu	12	F	2.7	4.5	8.3	5.5	2.0	0.9	1.7	20.0
Controls											
13	Asn91Ser	11	M	-1.1	—	5.9	4.8	1.3	0.4	1.2	3.9
14	Glu57Lys	11	M	0.3	—	15.5	4.1	2.9	0.8	1.1	3.5
15	Asp53Gly	12	M	0.9	—	ND	ND	ND	ND	1.2	4.2
16	Glu214Gly	13	M	1.6	—	15.0	5.7	3.8	1.1	1.4	21.4
17	Glu214Gly	12	F	0.1	—	4.2	4.7	0.9	0.8	1.5	8.6
18	Glu214Gly	11	F	3.9	—	23.2	5.1	5.2	1.3	1.1	40.8
19	Glu214Gly	12	M	-1.2	—	5.2	4.9	1.1	0.4	1.7	0.8
20	Ala195Thr	14	F	0.8	—	ND	ND	ND	ND	ND	16.8

M, male, F, female; age at obesity onset is defined as age with BMI above the 97th percentile; Ins T0, fasting insulinemia; Gly T0: fasting glucose; HOMA, homeostasis model assessment index defined as (fasting insulin level \times fasting glucose level) divided by 22.5; TG, triacylglycerol; Chol, total cholesterol; ?, data not available; ND, not determined.

Biological parameters were measured according to methods described in Materials and Methods section.

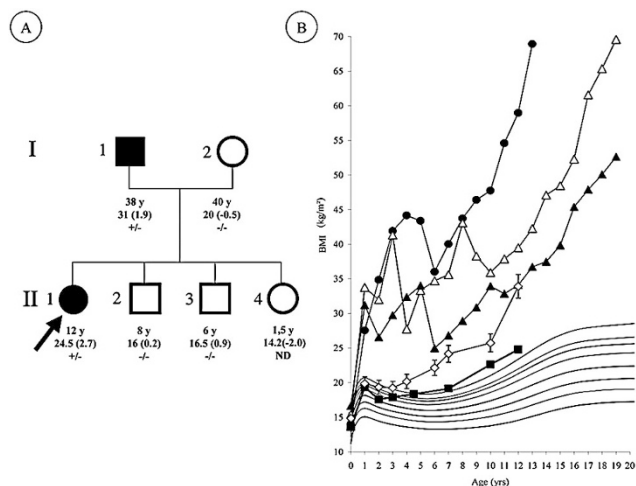


Figure 2. Pedigree, genotype, and clinical characteristics of the Phe144Leu POMC mutation carrier. (A) Pedigree and genotypes. The proband III is a 12-y-old female with a BMI of 22.5 kg/m² (BMI Z score = 2.7 SDs). She is heterozygous for a Phe144Leu (t > c 431) mutation. The mutated allele was transmitted by her father who is moderately obese (BMI, 31 kg/m² at age 38 y, BMI Z score = 1.9 SDs). Subjects I2 (BMI, 21 kg/m² at age 40 y, BMI Z score = -0.5 SD), II2 (age, 8 y; BMI Z score = 0.2 SD) and II3 (age, 6 y; BMI Z score = 0.9 SD) are nonobese carriers of two normal alleles. Obese individuals (solid circles and squares). Age, BMI Z score, and genotype (+, normal; -, mutated) are indicated on the pedigree; ND, not determined. (B) BMI curves of the proband (HetPOMC), six heterozygous MC4R mutation carriers (HetMC4R), and three homozygous null leptin receptor mutants (LepR 1, 2, 3). HetPOMC (solid squares); LepR 1 (solid circles); LepR 2 (open triangles); LepR 3 (solid triangles); HetMC4R (open diamonds).

mutation carriers (5,17). Precise chemical analysis measuring hair melanin peptide and skin pigmentation would be necessary to firmly conclude. We also observed normal results in

hypothalamic-pituitary-adrenal axis explorations in the Phe144Leu carrier, which could be explained by the fact that the mutation does not alter the effect of ACTH on melanocortin 2 receptor. This is in agreement with previous findings observed in heterozygous carriers of POMC mutations (5,12,17,18). However, this would need further *in vitro* functional investigation.

The BMI curve of this subject shows several interesting findings. Obesity starts as early as in the MC4R mutant carriers, suggesting a very early effect of the functionally relevant Phe144leu mutation on weight gain after birth. However, it is less severe over time. This is not due to a change in diet because both MC4R and POMC mutated subjects did not undergo any dietary intervention until the age of 10 y. This observation suggests that, in humans, deleterious heterozygous mutations in the α MSH domain of the POMC gene might lead to a less severe obese phenotype than heterozygous mutations in MC4R gene. While the central melanocortin system is intolerant to the loss of a single MC4R allele, it appears to be able to tolerate the loss of one copy of the POMC gene, except for mutations in β MSH. The Phe144Leu mutation in α MSH, as the one recently reported by Lee *et al.* (18), appears to have only a moderate effect on the obese phenotype when compared with the effect of β MSH mutations. Our findings provide additional support for a more critical role of β MSH than of α MSH in the control of human energy balance (12,17,18). Because β MSH is present in the human hypothalamus (25) and seems to have higher affinity for the hMC4R (26), it is likely to be the more physiologically relevant endogenous ligand for the hMC4R (when compared with α MSH). The obese phenotype associated with the POMC

Table 3. Endocrine characteristics of the proband with the Phe144Leu mutation

	Phe144Leu	Normal range
UFC ($\mu\text{g}/24\text{ h}$)	17.5	23 ± 11
CRF test		
Cortisol (ng/mL) (0–60 min)	53–146	104 ± 48 – 184 ± 29
ACTH (pg/mL) (0–60 min)	25–76	<40 – 77 ± 27
LPH (pg/mL) (0–60 min)	124–218	87 ± 38 – 167 ± 49
TSH ($\mu\text{U}/\text{mL}$)	3.0	1.78 (0.2–3.5)
Free T_4 (pg/mL)	7.2	8.5 (5.8–11.5)
OGTT		
Glucose (mmol/L) (0–30–120 min)	5.0–7.2–6.5	T0: 3.3–5.8; T120: <7.8
Insulinemia ($\mu\text{UI}/\text{mL}$) (0–30–120 min)	8.3–32.2–16.9	T0: <5

UFC, urinary free cortisol; CRF, corticotropin releasing factor; LPH, lipoprotein hormone; OGTT, oral glucose tolerance test.

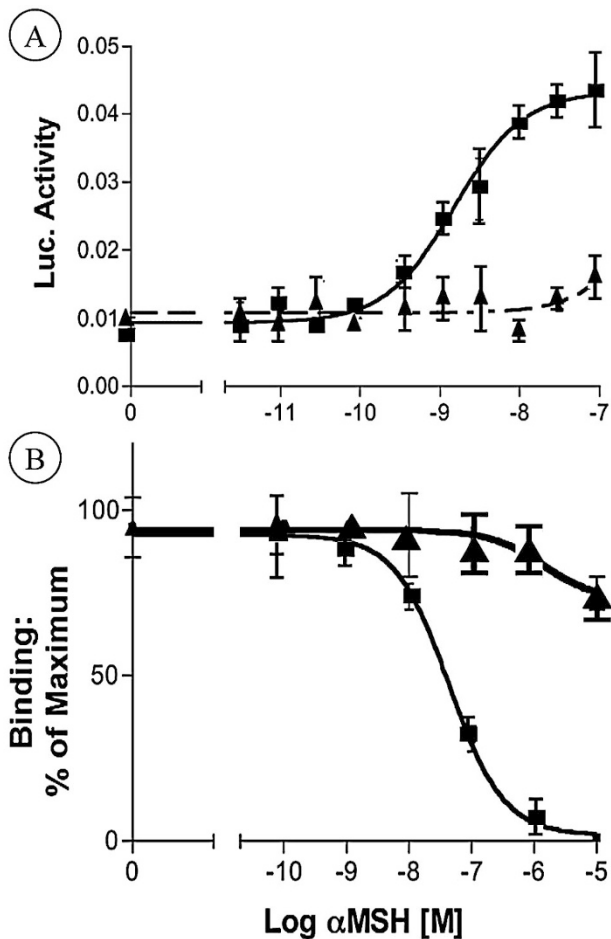


Figure 3. Impaired activation and binding of MC4R by the αMSH product of Phe144Leu POMC. Leu144 αMSH (Leu at position 7 of αMSH corresponding to the mutated POMC allele) and Phe144 αMSH (Phe at position 7 corresponding to wild type) were synthesized and purified by high-performance liquid chromatography. (A) MC4R activity in response of Phe144 αMSH or Leu144 αMSH . Data points represent means of five determinations and error bars indicate SDs. The $\text{EC}_{50} \pm \text{SDs}$ for αMSH activation is $1.5 \pm 0.7\text{ nM}$. (B) Competition binding assay of Phe144 αMSH or Leu144 αMSH on the human MC4R. Each point is the mean of triplicate values and error bars indicate SDs. The IC_{50} for Phe144 αMSH in this experiment is $29.6 \pm 7.52\text{ nM}$. Phe144 αMSH (solid squares); Leu144 αMSH (solid triangles).

mutation reported here could also be modulated by other variants in modifier genes, intrinsic and/or extrinsic to the melanocortin pathway. Cosegregation analysis also showed that the obese father carrying the mutation has a comparatively less

pronounced form of obesity. Gene-environment interactions are a plausible explanation, as the younger generation is growing up in a much more obesogenic environment compared with the older generation. This family pedigree with the POMC mutation further illustrates the complex nature of human obesity when variant alleles in certain “thrifty” genes might interact together to favor weight gain in a predisposing environment.

In our study, the frequency of POMC gene variations was not significantly different between obese and control children. In contrast to MC4R mutations that are significantly more frequent in obese populations (27,28), it suggests that the implication of POMC mutations in the development of obesity remains to be discussed. Although carriers of POMC mutations have been described in several studies (4,5,11–13,16–18), the effect of the mutations on the obesity phenotype appears to be variable and the study of the functional consequences of POMC mutations are still rare but necessary to demonstrate their pathogenic role. Up to now, only a few POMC mutations may be considered a cause of monogenic obesity (4,12,17,18).

In conclusion, we found a similar frequency of POMC mutations in lean and obese children. Only rare deleterious mutations, such as the Phe144Leu described here, might contribute to early-onset obesity (11–13,16–18). In addition to previous studies, our findings confirm the less critical role of the POMC gene mutations in the complex determinism of human obesity when compared with MC4R mutations that are involved in 2%–3% of human obesity (27,28). In addition, they may confirm the more critical role of βMSH than αMSH in the control of human energy balance because mutations in αMSH appear to have only a moderate effect on the obese phenotype when compared with the effect of βMSH mutations and support the physiologic role of the melanocortin pathway in human weight regulation.

Acknowledgments. The authors are grateful to the medical staff of Pediatric Departments (Trousseau, Caen, Margency) for patient recruitment and to Pr. Yves Lebouc and his staff for endocrine explorations. They also thank the ATC Nutrition INSERM. Investigations were performed in the Center of Research on Human Nutrition (CRNH).

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