

Melanin-Concentrating Hormone Receptor Mutations and Human Obesity: Functional Analysis

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Abstract

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Melanin-concentrating hormone (MCH), a neuropeptide highly expressed in the lateral hypothalamus, has an important role in the regulation of energy balance and body weight in rodents. We examined whether mutations in the two known MCH receptors might be associated with obesity-related phenotypes in humans. Among 106 subjects with severe early onset obesity and a history of hyperphagia, we found two missense variants in *MCHR1*: Y181H and R248Q. Neither of these was found in 192 normal weight controls. R248Q cosegregated with obesity across two generations; family data were unavailable for Y181H. When expressed in HEK293 cells, R248Q showed no evidence of constitutive activation or ligand hypersensitivity for extracellular signal-regulated kinase phosphorylation. In addition, R248Q showed no enhanced suppression of cAMP generation. Two common single-nucleotide polymorphisms were found to be in linkage disequilibrium: g.-114A>G and c.39C>T. No association between either of these single-

nucleotide polymorphisms and obesity-related phenotypes was found among a population cohort of 541 whites. Only two rare noncoding variants were found in *MCHR2*. In conclusion, mutations in the MCH receptors are not commonly found in humans with severe early onset obesity. Clarification of the relationship of these variants to obesity must await study in other populations and/or in genetically modified mice.

Key words: *MCHR1*, *MCHR2*, association study, ERK phosphorylation, G-protein-coupled receptors

Melanin-concentrating hormone (MCH)¹ is predominantly expressed in the zona incerta and lateral hypothalamus, both of which are regions implicated in the regulation of feeding behavior and energy homeostasis. Central administration of MCH increases feeding in rats, and levels of MCH are upregulated in both leptin-deficient *ob/ob* mice and in fasted control mice (1). These data implicate MCH in the hypothalamic feeding circuit downstream of leptin. Mice lacking the gene for pro-MCH are hypophagic and lean, whereas mice overexpressing MCH in the lateral hypothalamus are hyperphagic and develop obesity (1,2). Two MCH receptors have been identified to date (3–7). Both are G-protein-coupled receptors that activate multiple second messenger systems and are expressed in a number of brain areas including olfactory and limbic structures, the ventromedial nucleus of the hypothalamus, and specific brainstem nuclei. Signaling through the MCH receptor type 1 plays an important role in mediating MCH's effects on energy balance, as shown by the fact that *MCHR1* knockout mice are lean and hyperactive (8,9). *PMCH* $-/-$; *ob/ob* double-null

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¹ Nonstandard abbreviations: MCH, melanin-concentrating hormone; DHPLC, denaturing high-performance liquid chromatography; ERK, extracellular signal-regulated kinase; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism; SDS, standard deviation score.

Table 1. Sequence variants in human MCH receptors 1 and 2 in 106 unrelated subjects with severe early onset obesity

Gene	Variant	Coding change	Type of change	Allele frequency (%)
<i>MCHR1</i>	g.-214C>A	Noncoding		3.7
<i>MCHR1</i>	g.-114G>A	Noncoding		39.2
<i>MCHR1</i>	c.39T>C	Silent	None	42
<i>MCHR1</i>	c.541T>C	Y181H	Nonconservative	0.5
<i>MCHR1</i>	c.743G>A	R248Q	Nonconservative	0.5
<i>MCHR1</i>	g*48T>C	Noncoding		0.5
<i>MCHR2</i>	g.-28A>C	Noncoding		0.5
<i>MCHR2</i>	c.228T>C	Noncoding		0.5

The SNPs in *MCHR1* designated c.541T>C, c.743G>A, and g*48T>C were found once each, in a heterozygous state. The same is true for the SNPs in *MCHR2*, designated g.-28A>C and c.228T>C.

mice show a marked increase in energy expenditure and improvements in several metabolic parameters when compared with *ob/ob* controls (10). The function of the MCH receptor type 2 is obscure because no ortholog exists in mice (11).

In the last 7 years, we and others have identified several single gene defects causing severe human obesity. Many of these defects have been in molecules identical or similar to those identified as a cause of obesity in rodents, and the further molecular and phenotypic characterization of these mutations has highlighted the importance of signaling molecules such as leptin and the melanocortins in the regulation of appetite and weight in humans (12–15). We have established a cohort of subjects with extreme obesity of prepubertal onset that has been particularly valuable in the identification of such monogenic defects. We have utilized this cohort to search for genetic defects in the MCH receptors that might lead to severe obesity. Having found common single-nucleotide polymorphisms (SNPs) in the *MCHR1* gene, we have also tested the association of these variants with obesity-related traits in a more general population. In this study, we examined 106 subjects with a history of hyperphagia and severe, early onset obesity for mutations in *MCHR1* and *MCHR2* using denaturing high-performance liquid chromatography (DHPLC) and further analyzed abnormal conformers by direct sequencing.

Six sequence variants were identified in the *MCHR1* gene (Table 1), two of which were nonconservative amino acid substitutions (R248Q and Y181H, see Figure 1A). Each of these was found as a heterozygous change in a single subject. Both R248Q and Y181H alter residues that are conserved in *MCHR1* across all mammalian species characterized to date, and both result in nonconservative amino acid changes. Arginine 248 lies in the second intracellular loop of the receptor, and tyrosine 181 lies in the fourth

transmembrane domain (Figure 1B). R248Q was identified in a severely obese 14-year-old white boy [BMI standard deviation score (SDS) = 3.41] and in his obese mother (BMI SDS = 2.1) but not in his lean father and sibling (Figure 1C). Y181H was identified in a severely obese boy of South Asian origin (BMI SDS = 4.62). Additional family members were unavailable for study. Neither of these variants was found in 192 British white control subjects or in 70 South Asian (Punjabi) controls.

To examine the functional consequences of the R248Q mutant, which appeared to segregate with obesity in a single pedigree, we generated HEK293 cells stably expressing mutant or wild-type receptors. Extracellular signal-regulated kinase (ERK) phosphorylation basally and in response to MCH was examined by Western blotting with a phospho-specific antibody. Phospho-ERK levels were not significantly different between mutant and wild-type cell lines either basally or after MCH stimulation (Figure 2A and 2B). We proceeded to study another known effect of the active MCH1 receptor, namely its ability to suppress cAMP generation. Endogenous cAMP levels were stimulated using forskolin, and the abilities of the mutant and wild-type *MCHR1* to suppress forskolin-stimulated cAMP levels were compared. No significant differences were observed between the mutant and wild-type receptors (Figure 2C).

Of the noncoding and synonymous SNPs in *MCHR1*, two (c.39T>C and g.-114G>A) were common. Both were in Hardy-Weinberg equilibrium, and the two were in tight linkage disequilibrium with each other. We examined whether the *MCHR1* SNPs were associated with obesity and obesity-related phenotypes (fasting insulin, glucose, leptin, and triglycerides) in a population cohort of 541 unrelated United Kingdom white subjects (Table 2). Valid genotypes were obtained for SNP g.-114G>A and SNP c.39T>C from 510 and 518 individuals, respectively, and for both

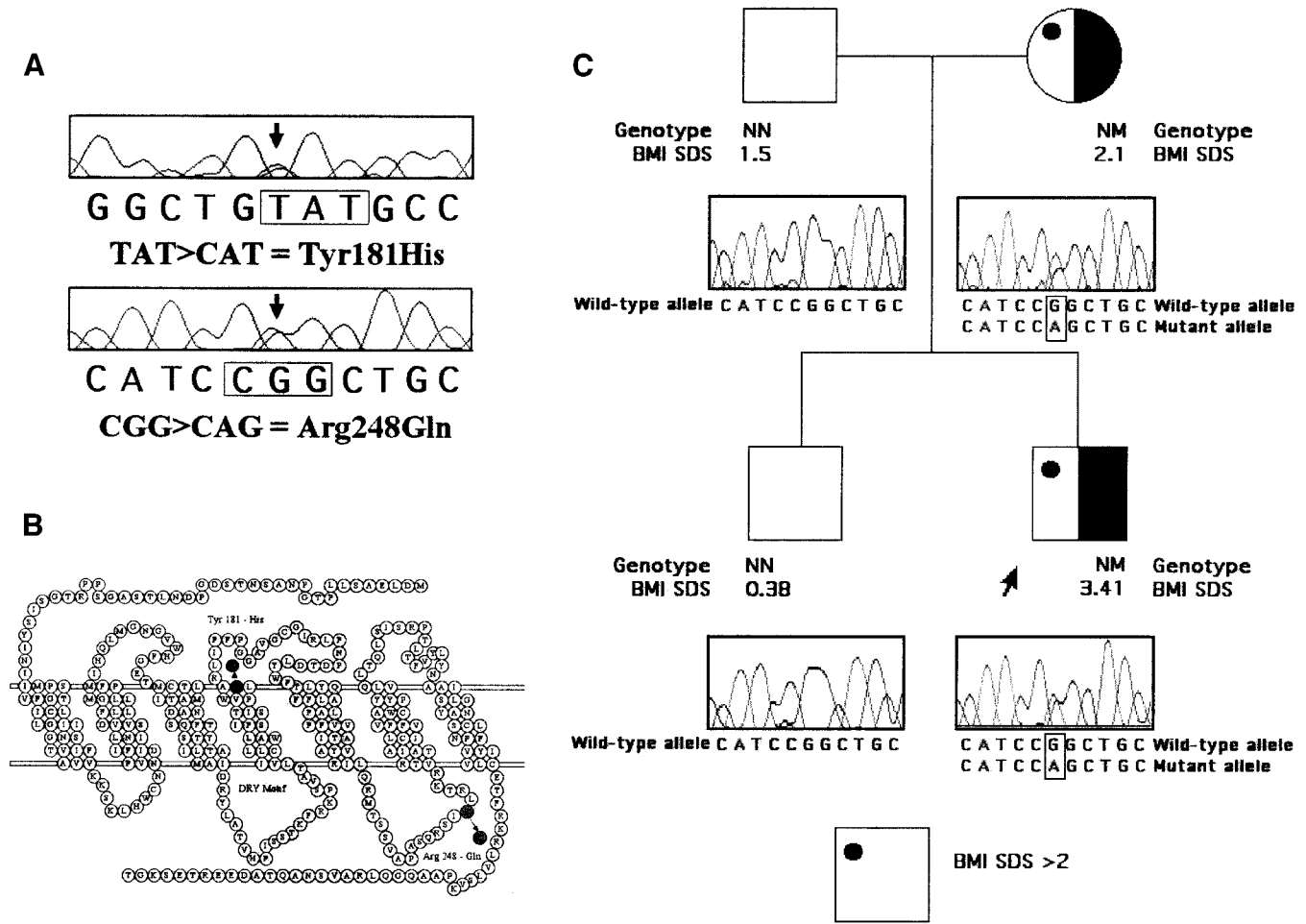


Figure 1: (A) Sequence chromatograms of nonconservative mutations in the *MCHR1* gene. Each mutation was discovered in the heterozygous state in one patient with severe early onset obesity. BMI SDS scores for the probands at the time of referral were 4.62 for Y181H and 3.41 for R248Q. (B) Schematic diagram of the human *MCHR1* receptor. The DRY motif is shown, and mutations described in this study are shown with arrows. The structure of the transmembrane domains is based on modeling information from the G-protein-coupled receptor database (available at <http://www.gpcr.org/7tm/>). (C) Pedigree for the family of the R248Q proband. BMI SDS scores are given below the genotype (NN, homozygous normal; NM, heterozygous). Individuals with a BMI SDS > 2 are indicated with a dot. The proband is identified by an arrow.

SNPs from 487 subjects. Estimated haplotype frequencies were 60.8% GT, 37.7% AC, and 1.5% GC. The AT haplotype was not observed. No significant associations were observed between the two SNPs and any of the anthropometric or biochemical parameters listed, nor were associations observed with any of the haplotypes. Haplotype association data are shown in Table 2 for the two common haplotypes, GT and AC. Study of the *MCHR2* gene revealed only two rare noncoding variants, which were not pursued further (Table 1).

Physiological and genetic studies in rodents have clearly implicated MCH as an orexigenic peptide neurotransmitter mediating its effects on energy balance through the *MCHR1* receptor. However, to date, little or no information is available regarding genetic variation in the *PMCH* or *MCHR1*

genes in relation to energy balance in humans. Furthermore, because a second subtype of the MCH receptor exists in humans but not in rodents, it is only through human studies that insights into the latter's function are likely to be obtained.

In this study, the first systematic examination, to our knowledge, of the two MCH receptor genes in relation to human obesity, we found two individuals with rare nonconservative missense mutations in *MCHR1*. Rare mutations in extreme human phenotypes have, in the past, been very valuable in highlighting key regulatory molecules important for human energy balance. However, in this instance, it is not clear whether these variants are likely to be pathogenic or not. In the case of Y181H, no other family members were available for study. In the case of R248Q, the limited family

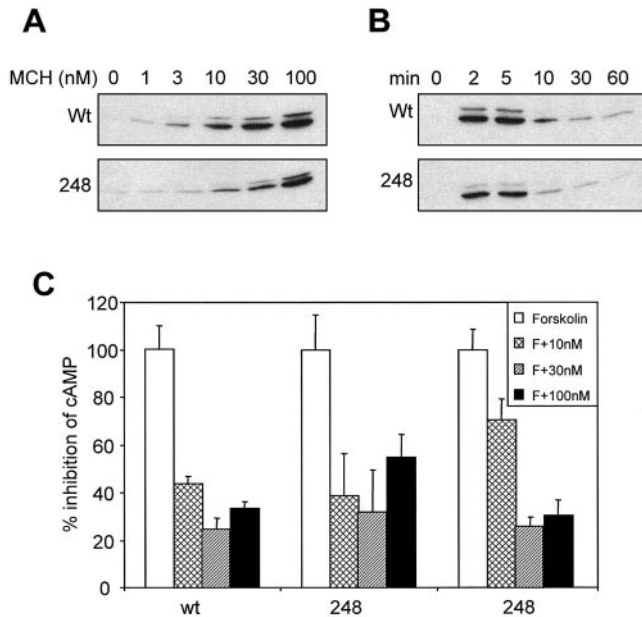


Figure 2: Functional analysis of stable HEK293 cell lines expressing wild-type (wt) and mutant R248Q MCH receptors. (A) Dose response of ERK activation with increasing concentration of MCH. Wt and R248Q clones were stimulated for 2 minutes with the indicated concentration of MCH, and phosphorylation of ERK was detected. (B) Time course of ERK activation in response to 100 nM MCH. Maximal activation is seen 2 minutes after MCH addition and a decrease to background levels within 10 minutes. (C) Percentage inhibition of forskolin-stimulated cAMP levels (1 μ M forskolin) by increasing concentration of MCH (10, 30, and 100 nM). One wild-type and two mutant cell lines are shown. cAMP levels in the presence of forskolin were set to 100% for each cell line.

information available did support a pathogenic role. However, we were unable to demonstrate any functional differences between wild-type and mutant receptors. Knowledge of the signaling pathways downstream of *MCHR1* is limited, however, and we may not have explored the most relevant second messengers. In addition, if a mutation in *MCHR1* were likely to cause obesity, one would speculate that it would do so by inducing constitutive activation or hypersensitivity to ligand. These effects might be quite subtle and not readily observable in artificial cellular systems with high levels of receptor expression. Furthermore, pathogenic mutations in *MCHR1* may present with a more complex phenotype than hyperphagia alone because *MCHR1* knockout mice are lean and hyperactive (8). It will be most interesting to see if this mutation is found in other populations of severely obese individuals.

We identified two common SNPs in *MCHR1*, but neither of these was found to be associated with any obesity-related trait in a population of 500 whites. This, of course, does not exclude the possibility that other coding or intronic variants in this gene or in the promoter region might be associated

with such phenotypes. Our examination of *MCHR2* was relatively uninformative for population genetics because only two very rare SNPs were detected.

In conclusion, we have conducted one of the first systematic examinations of the MCH receptor genes in relation to human obesity. Although mutations in these genes were not commonly found in subjects with severe early onset obesity, we did detect two rare missense variants in *MCHR1* in severely obese hyperphagic probands. However, their causative relationship with obesity is as yet unclear. Further clarification will require study in larger populations of obese subjects and their families and/or their reconstitution in genetically modified mice.

Research Methods and Procedures

Polymerase Chain Reaction (PCR), DHPLC, and Sequencing

Human genomic DNA was isolated from whole blood using a QIAamp blood kit (QIAGEN, London, United Kingdom) and amplified with NRich (Genetix, Hampshire, United Kingdom). The two coding exons and 5'-untranslated region of the *MCHR1* gene were amplified with four PCR reactions. Five PCR reactions were designed to amplify the five coding exons from the human *MCHR2* gene (primers listed in Table 3). PCR was carried out under standard conditions, and products were analyzed by DHPLC according to the WAVE protocol (Transgenomic, Omaha, NE). Products which generated abnormal DHPLC patterns were sequenced using BigDye terminator chemistry (Perkin-Elmer, Foster City, CA) and analyzed on an ABI 377 automated DNA sequencer (Perkin-Elmer). Typing of SNPs in the Ely cohort was done by matrix-assisted laser desorption ionization/time of flight mass spectrometry at the Sanger Institute (Hinxton, United Kingdom).

Study Populations

Subjects with severe obesity of early onset (<10 years of age) have been recruited to the United Kingdom Genetics of Obesity Study. For this study, unrelated subjects were selected based on a history of hyperphagia. The mean BMI SDS of probands is 4.2 ± 0.8 .

The MRC Ely Study is a prospective population-based cohort study of the etiology and pathogenesis of type 2 diabetes and related metabolic disorders in white adult subjects 40 to 65 years old from Ely, Cambridgeshire, United Kingdom (16). In addition to anthropometric measurements, data on biochemical variables such as insulin and leptin are available, as are measurements of body composition and resting energy expenditure.

Statistical Analysis

Linkage disequilibrium test and haplotype analyses were performed using the expectation maximization algorithm.

Table 2. MCHR1 SNPs and obesity-related phenotypes in a United Kingdom white population

	g.-114G>A			c.39T>C			Haplotypes g.-114G>A + c.39T>C		
	G/G	G/A + A/A	T/T	T/C + C/C	GT/GT	GT/AC	AC/AC		
N (males/females)	99/104	120/187	98/96	127/197	93/93	90/137	29/45		
Age (years)	53.9	54.3	53.8	54.1	54.0	54.4	53.8		
BMI (kg/m ²)	26.0 ± 0.29	26.2 ± 0.24	26.1 ± 0.30	26.2 ± 0.23	26.1 ± 0.30	26.4 ± 0.27	25.7 ± 0.48		
Fasting insulin (pM)*	41.2 (38.7, 43.9)	39.9 (37.9, 42.1)	40.8 (38.2, 43.5)	40.1 (38.1, 42.2)	41.4 (38.7, 44.2)	40.4 (38.1, 43.0)	37.8 (34.0, 42.0)		
120 min insulin (pM)*	228 (208, 249)	231 (214, 248)	220 (201, 242)	230 (214, 247)	227 (207, 249)	237 (217, 258)	212 (183, 246)		
Leptin (ng/mL)*	7.17 (6.47, 7.95)	7.54 (6.95, 8.18)	7.01 (6.31, 7.80)	7.59 (7.01, 8.22)	7.09 (6.36, 7.91)	7.83 (7.12, 8.60)	6.59 (5.57, 7.79)		
Triglycerides (mM)*	1.25 (1.18, 1.32)	1.25 (1.19, 1.31)	1.24 (1.17, 1.32)	1.24 (1.19, 1.30)	1.26 (1.18, 1.33)	1.25 (1.19, 1.32)	1.22 (1.11, 1.34)		
NEFA AUC (mM/h)	0.50 ± 0.01	0.52 ± 0.01	0.51 ± 0.01	0.51 ± 0.01	0.51 ± 0.02	0.53 ± 0.01	0.49 ± 0.02		
Body fat (%)	30.6 ± 0.36	30.7 ± 0.30	30.7 ± 0.37	30.6 ± 0.29	30.7 ± 0.38	31.0 ± 0.35	29.9 ± 0.60		
Resting energy expenditure (KJ/min)	5.90 ± 0.08	5.87 ± 0.06	5.93 ± 0.08	5.89 ± 0.06	5.89 ± 0.08	5.84 ± 0.07	5.94 ± 0.13		

NEFA AUC, nonesterified fatty acids (area under the curve). Percentage body fat is determined by DXA scan. The natural logarithm of the biochemical variables have been used to normalize the data. Means are adjusted for age, sex, and BMI, except when the outcome is BMI itself. No significant differences were found among groups. Data are mean ± SE.

* Geometric mean (95% confidence interval).

Table 3. Primers for amplification and sequencing of the human *MCHR1* and *MCHR2* genes

Primer	Sequence	Position	Size(nt)
<i>MCHR1 (GPR24)</i>			
GPR24X1FOR4	5'-ACAGCCTGGGACTGAAGAGG-3'	g.-267-g.-248	20
GPR24X1FOR1	5'-AGCTCAGCTCGGTTGTGG-3'	g.-42-g.-25	18
GPR24X1REV1	5'-GTGAAACCTTCCCATTTTCC-3'	IVS1+60-IVS1+41	20
GPR24X1REV2	5'-CTCAGGGGTGAAACCTTCC-3'	IVS1+67-IVS1+49	19
GPR24X25FOR1	5'-CAAAGCCCATGTCAAACAGC-3'	IVS1-45-IVS1-26	20
GPR24X25FOR2	5'-CCATGTCAAACAGCCAACG-3'	IVS1-39-IVS1-21	19
GPR24X25REV1	5'-GGAACCTTCGTGGAAGAGATGG-3'	c.463-c.443	21
GPR24X2MFOR1	5'-ACTTTGGGGAGACCATGTGC-3'	c.329-c.348	20
GPR24X2MFOR2	5'-ATGTGCACCCTCATCACG-3'	c.343-c.360	18
GPR24X2MREV1	5'-CGGCTGATGGACAACCTGG-3'	c.851-c.834	18
GPR24X23FOR1	5'-CCATCGCCATCTGTCTGG-3'	c.773-c.790	18
GPR24X23REV1	5'-AAAACCCCAAACCTGACTCC-3'	g.*193-g.*174	20
GPR24X23REV2	5'-AAACCCCAAACCTGACTCC-3'	g.*192-g.*174	19
RQMtag	5'-CAGCGCAGCATCCAGCTGCGGACAAAG-3'	c.730-c.756	27
RQRtag	5'-CTTTGTCCGCAGCTGGATGCTGCGCTG-3'	c.756-.730	27
<i>MCHR2 (GPR145)</i>			
MCHR2X1FOR1	5'-ACCCTGGGATCCTGTGTTC-3'	g.-158-g.-140	19
MCHR2X1FOR2	5'-ATACTCACCTGGGATCCTG-3'	g.-164-g.-145	20
MCHR2X1REV1	5'-TGCTACAGGGGAAAGGACTC-3'	IVS1+90-IVS1+71	20
MCHR2X1REV2	5'-GGGAGCTTTGACTGTAGTTTCC-3'	IVS1+44-IVS1+65	22
MCHR2X2FOR1	5'-GGCATTTCCTCTATGCATCTG-3'	IVS1-142-IVS1-122	21
MCHR2X2FOR2	5'-CAATGTCAATCCTCAGTTTCCTC-3'	IVS1-35-IVS1-13	23
MCHR2X2REV1	5'-TGGAATTGGAATGAGCTTGG-3'	IVS2+74-IVS2+55	20
MCHR2X2REV2	5'-ACTGGTGTGGAAATGGAATG-3'	IVS2+82-IVS2+62	21
MCHR2X3FOR1	5'-TCATGAACCTTTCATCCTTGG-3'	IVS2-73-IVS2-53	21
MCHR2X3FOR2	5'-TTCATCCTTGGAGCTCAGTG-3'	IVS2-63-IVS2-44	20
MCHR2X3REV1	5'-GGAGATACTGGCGAGAAGAGG-3'	IVS3+122-IVS3+102	21
MCHR2X3REV2	5'-GGCGAGAAGAGGATCCATAAG-3'	IVS3+113-IVS3+93	21
MCHR2X4FOR1	5'-AAAAGTCAAAGCCGATTC-3'	IVS3-83-IVS3-64	20
MCHR2X4FOR2	5'-CAAAGCCGATTCCTAATCC-3'	IVS3-76-IVS3-57	20
MCHR2X4REV1	5'-TCTGTGATTTCAACTCGTCAC-3'	IVS4+210-IVS4+188	23
MCHR2X4REV2	5'-CAACTCGTCACTGAGAGATTC-3'	IVS4+200-IVS4+178	23
MCHR2X5FOR1	5'-GCCCCATAATTTCTATGGTTG-3'	IVS4-56-IVS4-36	21
MCHR2X5FOR2	5'-GAACACATTGCCCCATAATTC-3'	IVS4-65-IVS4-44	22
MCHR2X5REV1	5'-ATCGGTACACCTGCCCTTTC-3'	g.*78-g.*59	20
MCHR2X5REV2	5'-GGTACACCTGCCCTTTCCTGA-3'	g.*75-g.*56	20

Mutagenic nucleotides are underlined (see Research Methods and Procedures).

One-way ANOVA tests for association with obesity, energy expenditure, and biochemical phenotypes were performed using the SAS for Windows System version 8.2 (SAS Institute Inc., Cary, NC). Means were adjusted for age, sex, and BMI, except when the outcome was BMI. Statistical associations were considered to be significant at $p < 0.05$.

Functional Studies

The *MCHR1* cDNA was cloned into a pcDNA3 vector using PCR. The cDNA was confirmed to be wild type by direct sequencing before site-directed mutagenesis. The R248Q mutation was introduced using the QuikChange site-directed mutagenesis kit (Stratagene, LaJolla, CA), and

the mutant cDNAs were sequenced to ensure that no mutations had been introduced by the amplification reactions. Finally, a FLAG epitope was inserted at the N-terminus of the cDNA by PCR mutagenesis, followed by confirmatory sequencing. HEK293 cells were transfected with Lipofectamine 2000 (Invitrogen, Carlsbad, CA), and stable transfectants were selected and maintained in the presence of 400 $\mu\text{g}/\text{mL}$ of Geneticin (Invitrogen) in Dulbecco's modified Eagle's medium-high glucose supplemented with 10% fetal bovine serum. Two wild-type and four mutant clones were characterized more extensively. cAMP assays were performed according to the manufacturer (Amersham, Piscataway, NJ). MCH and forskolin were purchased from Bachem (King of Prussia, PA) and Sigma (St. Louis, MO), respectively. P-ERK stimulation experiments and immunoblotting were performed as previously described (17).

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References

1. Shimada M, Tritos NA, Lowell BB, Flier JS, Maratos-Flier E. Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature*. 1998;396:670–4.
2. Ludwig DS, Tritos NA, Mastaitis JW, et al. Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. *J Clin Invest*. 2001;107:379–86.
3. Chambers J, Ames RS, Bergsma D, et al. Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1. *Nature*. 1999;400:261–5.
4. Saito Y, Nothacker HP, Wang Z, Lin SH, Leslie F, Civelli O. Molecular characterization of the melanin-concentrating-hormone receptor. *Nature*. 1999;400:265–9.
5. An S, Cutler G, Zhao JJ, et al. Identification and characterization of a melanin-concentrating hormone receptor. *Proc Natl Acad Sci U S A*. 2001;98:7576–81.
6. Hill J, Duckworth M, Murdock P, et al. Molecular cloning and functional characterization of MCH2, a novel human MCH receptor. *J Biol Chem*. 2001;276:20125–9.
7. Sailer AW, Sano H, Zeng Z, et al. Identification and characterization of a second melanin-concentrating hormone receptor, MCH-2R. *Proc Natl Acad Sci U S A*. 2001;98:7564–9.
8. Chen Y, Hu C, Hsu CK, et al. Targeted disruption of the melanin-concentrating hormone receptor-1 results in hyperphagia and resistance to diet-induced obesity. *Endocrinology*. 2002;143:2469–77.
9. Marsh DJ, Weingarh DT, Novi DE, et al. Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. *Proc Natl Acad Sci U S A*. 2002;99:3240–5.
10. Segal-Lieberman G, Bradley RL, Kokkotou E, et al. Melanin-concentrating hormone is a critical mediator of the leptin-deficient phenotype. *Proc Natl Acad Sci U S A*. 2003;100:10085–90.
11. Tan CP, Sano H, Iwaasa H, et al. Melanin-concentrating hormone receptor subtypes 1 and 2: species-specific gene expression. *Genomics*. 2002;79:785–92.
12. Montague CT, Farooqi IS, Whitehead JP, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature*. 1997;387:903–8.
13. Jackson RS, Creemers JW, Ohagi S, et al. Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet*. 1997;16:303–6.
14. Farooqi IS, Yeo GS, Keogh JM, et al. Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. *J Clin Invest*. 2000;106:271–9.
15. Challis BG, Pritchard LE, Creemers JW, et al. A missense mutation disrupting a dibasic prohormone processing site in pro-opiomelanocortin (POMC) increases susceptibility to early-onset obesity through a novel molecular mechanism. *Hum Mol Genet*. 2002;11:1997–2004.
16. Williams DR, Wareham NJ, Brown DC, et al. Undiagnosed glucose intolerance in the community: the Isle of Ely Diabetes Project. *Diabetes Med*. 1995;12:30–5.
17. Pissios P, Trombly DJ, Tzamelis I, Maratos-Flier E. Melanin-concentrating hormone receptor 1 activates extracellular signal-regulated kinase and synergizes with G(s)-coupled pathways. *Endocrinology*. 2003;144:3514–23.