Loss of Function of the Melanocortin 2 Receptor Accessory Protein 2 Is Associated with Mammalian Obesity

Masato Asai,[1,2] Shwetha Ramachandrappa,[3] Maria Joachim,[1] Yuan Shen,[1] Rong Zhang,[1] Nikhil Nuthalapati,[1] Visali Ramanathan,[1] David E. Strochlic,[1] Peter Ferket,[4] Kirsten Linhart,[1]* Caroline Ho,[1] Tatiana V. Novoselova,[5] Sumedha Garg,[3] Martin Ridderstråle,[6] Claude Marcus,[7] Joel N. Hirschhorn,[1,8] Julia M. Keogh,[3] Stephen O'Rahilly,[3] Li F. Chan,[5] Adrian J. Clark,[5] I. Sadaf Farooqi,[3]† Joseph A. Majzoub[1]†

Melanocortin receptor accessory proteins (MRAPs) modulate signaling of melanocortin receptors in vitro. To investigate the physiological role of brain-expressed melanocortin 2 receptor accessory protein 2 (MRAP2), we characterized mice with whole-body and brain-specific targeted deletion of *Mrap2*, both of which develop severe obesity at a young age. Mrap2 interacts directly with melanocortin 4 receptor (Mc4r), a protein previously implicated in mammalian obesity, and it enhances Mc4r-mediated generation of the second messenger cyclic adenosine monophosphate, suggesting that alterations in Mc4r signaling may be one mechanism underlying the association between *Mrap2* disruption and obesity. In a study of humans with severe, early-onset obesity, we found four rare, potentially pathogenic genetic variants in *MRAP2*, suggesting that the gene may also contribute to body weight regulation in humans.

Membrane-expressed G protein–coupled receptors (GPCRs) modulate cellular responses to numerous physiological stimuli. The melanocortin receptors (MCRs) are a subfamily of GPCRs that mediate signaling in response to the pro-opiomelanocortin–derived peptides, adrenocorticotropic hormone (ACTH), and α-melanocyte–stimulating hormone (αMSH) and their competitive antagonists, agouti and agouti-related protein. The MCRs mediate a diverse range of physiological functions: MC1R is involved in skin pigmentation, MC2R plays a critical role in the hypothalamic-pituitary-adrenal axis, MC3R and MC4R are involved in energy homeostasis, and MC5R is implicated in exocrine function (*1*).

There is increasing recognition that accessory proteins can modulate GPCR trafficking, as well as ligand binding and signaling (*2*). An accessory protein for MC2R, MC2R accessory protein (MRAP), is required for the trafficking of MC2R to the surface of adrenal cells and for signaling in response to ACTH (*3*, *4*). Loss of either MC2R or MRAP in humans causes severe resistance to ACTH, with resulting glucocorticoid deficiency (*5*, *6*).

All mammals have a paralogous gene, *MRAP2*, which, like *MC3R* and *MC4R*, is predominantly expressed in the brain (*7*), most prominently in the pons and cerebellum but also in regions involved in energy homeostasis, such as the hypothalamus and brainstem (fig. S1, A to C). Within the paraventricular nucleus of the hypothalamus (PVN), Mrap2 and Mc4r mRNAs are coexpressed in many cells (fig. S1D). We hypothesized that Mrap2 might modulate signaling through a MCR and potentially affect energy homeostasis. We therefore performed targeted deletion of *Mrap2* in mice using Cre-lox–mediated excision of the 100-bp exon 3 [which encodes the highly conserved transmembrane domain (*7*)] to create mice with normal levels of an mRNA predicted to encode a truncated protein that includes the first 55 amino acids of Mrap2, with the transmembrane domain replaced by 11 aberrant amino acids specified by the out-of-frame exon 4, followed by a stop codon (fig. S1, E to H). Normal levels of the mutant mRNA indicate preservation of Mrap2-containing neurons in null mice, although these neurons probably do not express the predicted mutant protein because mutant Mrap2 mRNA, but not

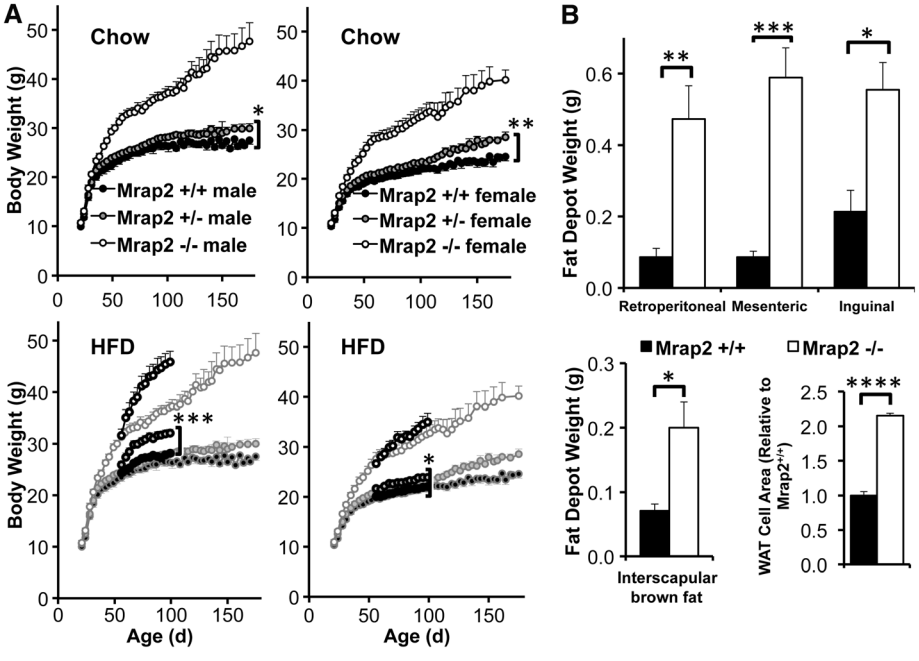


Fig. 1. Phenotype of *Mrap2*$^{-/-}$ mice. (**A**) Weight curves for *Mrap*$^{+/+}$ versus *Mrap*$^{+/-}$ versus *Mrap2*$^{-/-}$ mice on standard-chow (Chow, top: male $n = 9$ versus 28 versus 15 mice, female $n = 12$ versus 18 versus 10 mice) or high-fat diets (HFD; ages 56 to 95 days, bottom: superimposed on standard-chow curves: male $n = 10$ versus 8 versus 10 mice; female $n = 7$ versus 12 versus 7 mice). For both genders, the weight curves of *Mrap*$^{+/+}$ and *Mrap*$^{+/-}$ mice on standard chow differ significantly at older ages (161 to 175 days) and at younger ages (56 to 95 days) on a high-fat diet. *$P = 0.02$, **$P = 0.001$, ***$P = 0.0003$. (**B**) Fat depots on standard-chow diet. (Top) White adipose tissue (WAT) weights in *Mrap*$^{+/+}$ versus *Mrap2*$^{-/-}$ (males and females, ages 117 to 122 days, $n = 5$ versus 4 mice, respectively). (Bottom left) Brown adipose tissue (BAT) weight in *Mrap*$^{+/+}$ versus *Mrap2*$^{-/-}$ mice (males and females, ages 117 to 122 days, $n = 5$ versus 4 mice). (Bottom right) WAT cell size in in *Mrap*$^{+/+}$ versus *Mrap2*$^{-/-}$ mice (females, 50 cells counted from each mouse). *$P = 0.009$, **$P = 0.003$, ***$P = 0.0003$, ****$P < 0.00001$.

[1]Division of Endocrinology, Department of Medicine, Boston Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA. [2]Departments of Pathology, Endocrinology and Diabetes, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. [3]University of Cambridge Metabolic Research Laboratories and National Institute for Health Research (NIHR) Cambridge Biomedical Research Centre, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK. [4]Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC 27695, USA. [5]William Harvey Research Institute, Centre for Endocrinology Queen Mary, University of London Barts and The London School of Medicine and Dentistry, London EC1M 6BQ, UK. [6]Department of Clinical Sciences, Lund University, Malmö, Sweden, and Steno Diabetes Center, DK-2820 Gentofte, Denmark. [7]Department for Clinical Science, Intervention and Technology, Karolinska Institute, Division of Pediatrics, National Childhood Obesity Centre, S-141 86 Stockholm, Sweden. [8]Department of Genetics, Harvard Medical School and Broad Institute, Cambridge, MA 02142, USA.

*Present address: Department of Internal Medicine, KH Salem, University of Heidelberg, 69120 Heidelberg, Germany.
†Corresponding author. E-mail: joseph.majzoub@childrens.harvard.edu (J.A.M.); isf20@cam.ac.uk (I.S.F.)

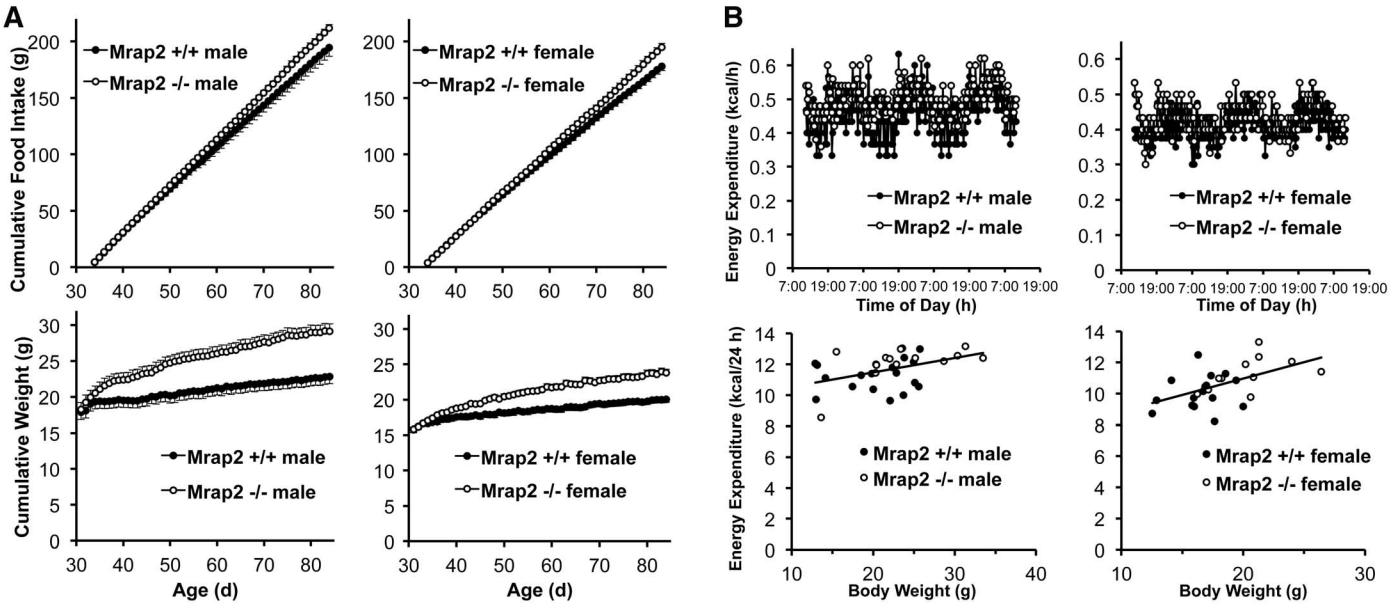
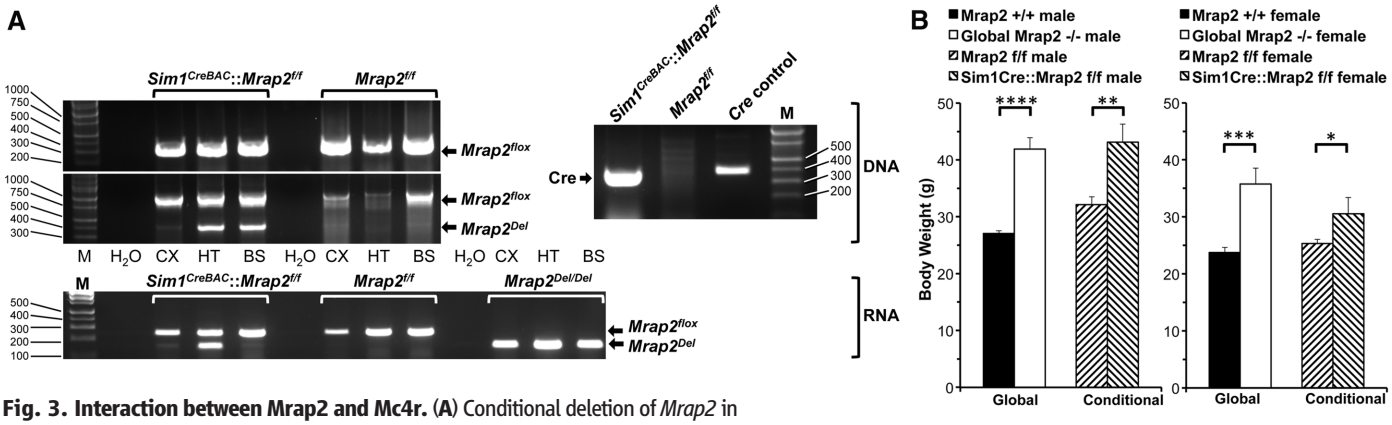


Fig. 2. Energy balance in *Mrap2*$^{-/-}$ mice. (**A**) Cumulative food intake (top) and weight (bottom) in ad libitum–fed *Mrap*$^{+/+}$ versus *Mrap2*$^{-/-}$ males (n = 10 versus 11 mice) and females (n = 11 versus 8 mice). (**B**) Energy expenditure in ad libitum–fed *Mrap*$^{+/+}$ versus *Mrap2*$^{-/-}$ mice. (Top) Continuous measurement over 3 days, males (n = 3 versus 4 mice), females (n = 4 versus 3 mice), ages 30 to 34 days. (Bottom) Body weight versus energy expenditure, integrated over 24 hours, males (n = 18 versus 14 mice, ages 30 to 45 days), females (n = 16 versus 11 mice, ages 30 to 42 days). Analysis with ANCOVA showed no differences between genotypes (males, P = 0.38; females, P = 0.67).



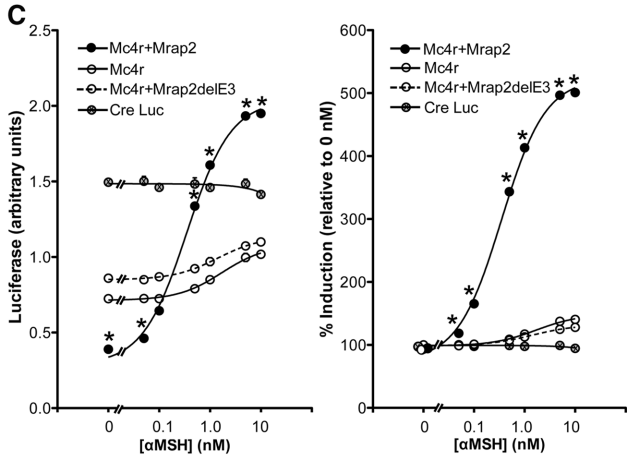


Fig. 3. Interaction between Mrap2 and Mc4r. (**A**) Conditional deletion of *Mrap2* in Sim1 neurons. (Top right) Cre DNA analysis by means of polymerase chain reaction (PCR). HT DNA from *Sim1*^{CreBAC}::*Mrap2*$^{f/f}$ mice contains *Cre* (374 bp), but from *Mrap2*$^{f/f}$ mice does not. Molecular weight marker (M) is shown on right (base pairs). (Top left) *Mrap2* DNA analysis in *Sim1*^{CreBAC}::*Mrap2*$^{f/f}$ and *Mrap2*$^{f/f}$ mice by means of PCR. Both genotypes contain floxed, intact *Mrap2* DNA in CX, HT, and BS (314 bp in top electropherogram, and 1013 bp in bottom electropherogram, and molecular weight markers on left). Only *Sim1*^{CreBAC}::*Mrap2*$^{f/f}$ mice contain *Mrap2*^{Del} (400 bp, bottom electropherogram), and only in HT and BS, but not in CX, which is consistent with fluorescent reporter data (fig. S3A). No PCR products are present without added DNA (H_2O). (Bottom) Mrap2 mRNA expression in *Sim1*^{CreBAC}::*Mrap2*$^{f/f}$ and *Mrap2*$^{f/f}$ mice by means of reverse transcriptase (RT)–PCR. Both genotypes express floxed, intact Mrap2 mRNA in CX, HT, and BS (247 bp). Only *Sim1*^{CreBAC}::*Mrap2*$^{f/f}$ mice express Mrap2^{Del} mRNA (147 bp), and only in HT. Global *Mrap2*$^{Del/Del}$ mice express Mrap2^{Del} mRNA in all three sites. (**B**) Body weights of *Mrap2*$^{+/+}$ (male n = 6 mice, female n = 11 mice), *Mrap2*$^{-/-}$ (male n = 11 mice, female n = 7 mice), *Mrap2*$^{f/f}$ (male n = 8 mice, female n = 12 mice), and conditional *Sim1*^{CreBAC}::*Mrap2*$^{f/f}$ (male n = 8 mice, female n = 7 mice) mice, all age 133 days. *P = 0.04, **P = 0.007, ***P = 0.0002, ****P < 0.0001. (**C**) Effect of Mrap2 on Mc4r signaling. (Left) Level of cAMP reporter activity (CRE Luc) in CHO cells alone or cotransfected with *Mc4r*, with or without *Mrap2* or the *Mrap2* knockout construct, *Mrap2delE3*, 5 hours after exposure to 0 to 10 nM αMSH (n = 3 mice per group). (Right) cAMP activity of these same constructs, expressed as percent induction after 0 to 10 nM αMSH, relative to 0 nM αMSH. *P < .0001, Mc4r+Mrap2 versus Mc4r at same [αMSH], by means of analysis of variance. For most data points, error bars are obscured by symbols.

protein, is present in cells transfected with the same *Mrap2* mutant construct used to create the null mice (fig. S1I).

Mrap2-null mice appeared normal at birth, with normal weight gain and post-weaning food intake during early life (0 to 32 days and 23 to 32 days, respectively), although young $Mrap2^{-/-}$ male mice trended toward greater weight and food intake with advancing age (fig. S1J). However, null mice of both genders gradually became extremely obese on a diet of regular chow ad libitum (figs. 1A and S2A). Heterozygous mice were significantly heavier than were wild-type animals on standard chow (160 to 175 days; males, $Mrap2^{+/+}$ 26.0 ± 0.4 g, $Mrap2^{+/-}$ 29.9 ± 0.9 g; females, $Mrap2^{+/+}$ 24.5 ± 0.9 g, $Mrap2^{+/-}$ 28.1 ± 0.7 g), and at younger ages (56 to 95 days) on a high-fat diet (Fig. 1A). In addition, $Mrap2^{-/-}$ mice had increased length (fig. S1K) and percent of weight due to fat and decreased percent of weight due to lean mass (fig. S1L). Both genders of $Mrap2^{-/-}$ mice had increased visceral adiposity, greater than twice the normal white adipose tissue cell size, enlarged brown adipose tissue depots, normal liver histology on a regular chow diet, but much greater hepatic steatosis as compared with those of wild-type mice on a high-fat diet (Fig. 1B and fig. S2, A and B). Adult *Mrap2*-null mice had, as expected, elevated leptin concentrations corresponding to their increased fat mass, which normalized with diet-induced weight normalization (fig. S2C). Obese adult mice had normal fasting insulin (fig. S2D) and normal tolerance to intraperitoneal glucose injection (fig. S2E). Mrap2 has been postulated to play a role in the adrenal response to ACTH (*8*). We therefore measured diurnal rhythmicity and stress responsiveness of the adrenal axis in *Mrap2*-null mice, which were normal (fig. S2F). Thyroid hormone levels were also normal (table S1). Epinephrine and norepinephrine excretion were reduced in male $Mrap2^{-/-}$ mice only (fig. S2G), but Ucp1 mRNA concentrations increased appropriately in both genders of null mice after exposure to 4°C for 18 hours (fig. S2H). Hypothalamic Agrp mRNA concentration was reduced in *Mrap2*-null mice, whereas Pomc mRNA was normal (fig. S2I).

To characterize the mechanisms underlying the obesity in these mice, we measured food intake under a variety of conditions. At 42 (fig. S2J) and 84 (fig. S2K) days of age, when $Mrap2^{-/-}$ mice were clearly overweight, no difference in food intake was detected between the two genotypes when analyzed over a 4-day interval. Obesity was not caused by more efficient absorption of calories in null mice (fig. S2L). Only when monitored daily over 50 days (ages 34 to 84 days) was a subtle increase in cumulative food intake discernable in the null animals (Fig. 2A), with the onset of obesity preceding hyperphagia (Fig. 2A and fig. S2M). To further understand the contribution of hyperphagia to obesity in $Mrap2^{-/-}$ mice, we limited their food intake to that amount consumed by their normal siblings (pair feeding). Even when fed the same amount of chow, null mice gained more weight than did wild-type mice (fig. S2, N and O). Only when the amount of food intake in null mice was further restricted to 10% (females) and 13% (males) less than that of wild-type mice was there equivalent weight gain (fig. S2P) in the two genotypes. To determine whether the late-onset hyperphagia in $Mrap2^{-/-}$ mice (Fig. 2A) could simply be the consequence of an increased body mass at this older age caused by a separate metabolic defect, we switched null mice to ad libitum access to chow after 40 days of restricted feeding (fig. S2P, upward arrow). During the first 24 hours of ad libitum feeding, food intake almost doubled in null mice (from 2.9 ± 0.1 to 5.6 ± 0.5 g/day in males, and from 2.8 ± 0.1 to 5.3 ± 0.2 g/day in females), with a corresponding marked increase in body weight. Thus, hyperphagia develops in an age-dependent manner in older mice, independent of body weight. Consistent with this, young (age 38 to 45 days) $Mrap2^{-/-}$ mice had an intact anorectic response to the MCR (Mc4r and Mc3r) agonist, MTII (fig. S2Q), corresponding to their normal ad libitum food intake at this age.

We hypothesized that young $Mrap2^{-/-}$ mice might display abnormal energy expenditure because obesity develops early during ad libitum feeding before the onset of hyperphagia, persists in mutant mice pair-fed to a normal dietary intake, and is abolished only by underfeeding. To explore this, we measured energy expenditure and respiratory exchange ratio (RER) with indirect calorimetry, as well as locomotor activity and core body temperature, in young (30 to 45 days of age) wild-type and *Mrap2*-null mice, just as their weights began to diverge (Fig. 2A). Surprisingly, the wild-type and mutant mice had indistinguishable 24-hour total energy expenditure, as analyzed by means of analysis of covariance (ANCOVA) (Fig. 2B) (*9*). There were also no differences between $Mrap2^{+/+}$ and $Mrap2^{-/-}$ mice in RER (fig. S2R), locomotor activity (fig. S2S), or core body temperature at 22°C (fig. S2T), with both genotypes exhibiting the expected increase in all three parameters during the active night period. After exposure to 4°C for 18 hours, null and wild-type mice became significantly hypothermic to the same extent (fig. S2T).

Because (i) MRAP is essential for signaling through MC2R (*3*, *4*), (ii) MRAP's paralog, *Mrap2*, is expressed principally in the brain, and (iii) Mc2r's paralog, Mc4r, has a key role in energy balance in Sim1-containing neurons (*10*), we asked whether deletion of *Mrap2* causes obesity in part by altering signaling through centrally expressed Mc4r. We created a $Sim1^{Cre}$::$Mrap2^{flox/flox}$ mouse with conditional deletion of *Mrap2* exclusively in these neurons and expression of $Mrap2^{Del}$ mRNA only in hypothalamus and not cerebral cortex or brainstem (Fig. 3A and fig. S3A). Like global null mice, conditional mutants were similarly obese (Fig. 3B), and pair-feeding to a normal dietary intake only partially reversed their obesity (fig. S3B).

If Mrap2 facilitates the action of Mc4r, then Mc4r deficiency should create an equivalent or more severe obesity phenotype than does Mrap2 deficiency, depending on the degree to which Mrap2 loss interferes with Mc4r function. Supporting this, $Mrap2^{+/-}$ mice of both genders were less obese than either $Mc4r^{+/-}$ or doubly heterozygous mice (fig. S3C). The differences between $Mc4r^{+/-}$ and doubly heterozygous mice were not statistically significant, although the latter trended toward being heavier. Among homozygous knockouts, those with Mc4r deficiency alone were more obese than those with Mrap2 deficiency alone (fig. S3C). The mice in which *Mc4r* was knocked out were more obese than were mice with deletion of both *Mc4r* and *Mrap2* (in males, with a trend in females), suggesting that Mrap2 may promote weight gain through both Mc4r-dependent and -independent actions.

To determine whether mouse Mrap2 and Mc4r can interact directly, we coimmunoprecipitated transiently expressed, N-terminally Myc-tagged Mrap2 and N-terminally green fluorescent protein–tagged Mc4r in Chinese hamster ovary (CHO) cells (devoid of endogenous Mrap, Mrap2, and MCRs). We found that mouse Mrap2 and Mc4r interact (fig. S3D), which is consistent with previous data (*7*). We next investigated the impact of Mrap2 on Mc4r (Fig. 3C) and Mc3r (fig. S3E) signaling. The combined expression of Mc4r and Mrap2 in CHO cells suppressed basal cyclic adenosine monophosphate (cAMP)–dependent protein kinase (PKA) signaling compared with Mc4r alone (Fig. 3C, left), as previously reported with the human orthologs (*7*). But in contrast to that

Table 1. *MRAP2* variants detected in obese subjects and controls.

MRAP2 variant	Subjects with variant	Subject sex/ age/BMI/ BMI SDS*	Controls with variant	MAF†: European American	MAF†: African American	***PolyPhen-2 prediction‡
E24X	1/488	M/19/63/4.7	0/488	0.000% (0/8600)	0.000% (0/4406)	Damaging
N88Y	1/376	M/11/29.6/3.3	0/376	0.000% (0/8600)	0.000% (0/4406)	Possibly damaging
L115V	1/488	M/5/24/4.2	0/488	0.012% (1/8600)	0.000% (0/4406)	Benign
R125C	1/488	F/8/29/3.5	0/488	0.047% (4/8600)	0.045% (2/4406)	Possibly damaging

*Subject sex (male, M; female, F)/age (years)/body mass index (BMI) (kilograms per square meter)/standard deviation score (SDS). †MAF, minor allele frequency; available at the National Heart, Lung, and Blood Institute exome variant server: http://evs.gs.washington.edu/EVS. ‡PolyPhen-2; available at http://genetics.bwh.harvard.edu/pph2.

report (which used NDP-MSH), we found that αMSH caused a fivefold increase above basal PKA activity (Fig. 3C, right) compared with less than a twofold increase with Mc4r alone or Mc4r plus the *Mrap2*-null construct, *Mrap2delE3* (our in vitro model for in vivo disruption of *Mrap2*). The presence of Mrap2 increased signaling through Mc3r at the two highest αMSH doses (fig. S3E). These findings suggest Mrap2 may alter signaling through Mc4r and perhaps other receptors.

To investigate whether alterations in MRAP2 are associated with human obesity, we sequenced the coding region and intron/exon boundaries of *MRAP2* in obese and control individuals from the Genetics of Obesity Study (GOOS) cohort (*11*) and the Swedish obese children's cohort (*12*). Four rare heterozygous variants that were absent from cohort-specific controls and 1000 genomes (Table 1) were found in unrelated, nonsyndromic, severely obese individuals, with all but one variant in the C-terminal region of the protein (fig. S4). In three of these subjects, no pathogenic variants were found in the coding region or intron/exon boundaries of all known nonsyndromic human obesity genes (table S2). Only one of the variants (E24X) is clearly disruptive, and overall, few rare variants were found in the obese cohorts, indicating that if *MRAP2* mutations contribute to severe human obesity, they do so rarely.

We have found that global or brain-specific inactivation of Mrap2 causes obesity in mice and that rare heterozygous variants in *MRAP2* are associated with early-onset, severe obesity in humans. The mechanism (or mechanisms) by which Mrap2 exerts its effects on body weight regulation remain to be firmly established but likely involve altered signaling through Mc4r and perhaps other MCRs. Under conditions comparable with those we describe, in which Mrap2 greatly enhances cAMP signaling through Mc4r, Sebag *et al.* (*13*) have found that the zebrafish ortholog of Mrap2 (zMRAP2b) similarly affects zMC4R signaling. This evolutionary conservation, plus the extreme disease phenotype caused by loss of Mrap2 function, supports the importance of Mrap2 in vertebrate biology.

References and Notes

1. R. D. Cone, *Endocr. Rev.* **27**, 736–749 (2006).
2. D. L. Hay, D. R. Poyner, P. M. Sexton, *Pharmacol. Ther.* **109**, 173–197 (2006).
3. P. M. Hinkle, J. A. Sebag, *Mol. Cell. Endocrinol.* **300**, 25–31 (2009).
4. S. N. Cooray, A. J. Clark, *Mol. Cell. Endocrinol.* **331**, 215–221 (2011).
5. L. A. Metherell *et al.*, *Nat. Genet.* **37**, 166–170 (2005).
6. L. F. Chan, L. A. Metherell, A. J. Clark, *Eur. J. Pharmacol.* **660**, 171–180 (2011).
7. L. F. Chan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 6146–6151 (2009).
8. J. A. Sebag, P. M. Hinkle, *Sci. Signal.* **3**, ra28 (2010).
9. M. H. Tschöp *et al.*, *Nat. Methods* **9**, 57–63 (2012).
10. N. Balthasar *et al.*, *Cell* **123**, 493–505 (2005).
11. S. Farooqi, S. O'Rahilly, *Endocr. Rev.* **27**, 710–718 (2006).
12. L. E. Johansson *et al.*, *PLoS ONE* **4**, e5327 (2009).
13. J. A. Sebag *et al.*, *Science* **341**, XXXX (2013).

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Supplementary Materials

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Materials and Methods
Figs. S1 to S4
Tables S1 to S3
References (*14*–*19*)

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Developmental Control of the Melanocortin-4 Receptor by MRAP2 Proteins in Zebrafish

Julien A. Sebag,[1]* Chao Zhang,[1]* Patricia M. Hinkle,[2] Amanda M. Bradshaw,[1] Roger D. Cone[1]†

The melanocortin-4 receptor (MC4R) is essential for control of energy homeostasis in vertebrates. MC4R interacts with melanocortin receptor accessory protein 2 (MRAP2) in vitro, but its functions in vivo are unknown. We found that MRAP2a, a larval form, stimulates growth of zebrafish by specifically blocking the action of MC4R. In cell culture, this protein binds MC4R and reduces the ability of the receptor to bind its ligand, α–melanocyte-stimulating hormone (α-MSH). A paralog, MRAP2b, expressed later in development, also binds MC4R but increases ligand sensitivity. Thus, MRAP2 proteins allow for developmental control of MC4R activity, with MRAP2a blocking its function and stimulating growth during larval development, whereas MRAP2b enhances responsiveness to α-MSH once the zebrafish begins feeding, thus increasing the capacity for regulated feeding and growth.

The melanocortin-4 receptor (MC4R), a G protein–coupled receptor (GPCR), plays a central role in energy homeostasis (*1*–*4*) and somatic growth (*1*, *2*, *5*). Mutations in the gene encoding MC4R are the most common monogenic cause of severe early-onset obesity in humans (*1*). In the zebrafish, as in mammals, MC4R is prominently involved in the regulation of energy homeostasis and somatic growth (*6*). Dominant negative mutations in MC4R are a natural cause of increased growth rate and final size in some teleost species (*5*). An artificially induced increase in MC4R activity early in the development of the zebrafish embryo causes a decrease in growth, a decrease in growth hormone gene expression, and a compensatory increase in growth hormone–releasing hormone (*ghrh*) gene expression (*6*), thus providing quantitative assays for MC4R activity in vivo. The melanocortin receptors have been shown to interact with the melanocortin receptor accessory proteins MRAP1 and MRAP2 (*7*–*13*), which are single-transmembrane proteins that form unusual antiparallel homo- and heterodimers (*7*–*9*). Whereas MRAP1 is essential for adrenocorticotropic hormone receptor (MC2R) trafficking to the plasma membrane, ligand binding, and downstream signaling (*7*, *8*, *11*), the functions of MRAP2 remain unclear. In the zebrafish, MRAP2 exists in two isoforms, a and b (*14*). Here, we investigated the role of MRAP2a and MRAP2b in the regulation of MC4R activity in vivo in the zebrafish and in vitro in human embryonic kidney (HEK) 293T cells.

We first characterized the distribution and developmental expression kinetics of *mc4r*, *mrap2a*, and *mrap2b* gene expression in the zebrafish embryo at 1, 2, 3, or 4 days post-fertilization (dpf) by reverse transcription polymerase chain reaction (RT-PCR) (Fig. 1A). *mc4r* and *mrap2a* mRNA were detectable from 1 dpf and their expression increased every day until 4 dpf, whereas *mrap2b* was hardly detectable. To identify the larval tissue distribution of *mrap2* mRNAs, we performed whole-mount in situ hybridization on zebrafish embryos at 5 dpf. *mrap2a* was ubiquitously ex-

[1]Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA. [2]Department of Pharmacology and Physiology, School of Medicine and Dentistry, University of Rochester Medical Center, Rochester, NY 14642, USA.

*These authors contributed equally to this work.
†Corresponding author. E-mail: roger.cone@vanderbilt.edu

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Loss of Function of the Melanocortin 2 Receptor Accessory Protein 2 Is Associated with Mammalian Obesity

Masato Asai, Shwetha Ramachandrappa, Maria Joachim, Yuan Shen, Rong Zhang, Nikhil Nuthalapati, Visali Ramanathan, David E. Strochlic, Peter Ferket, Kirsten Linhart, Caroline Ho, Tatiana V. Novoselova, Sumedha Garg, Martin Ridderstråle, Claude Marcus, Joel N. Hirschhorn, Julia M. Keogh, Stephen O'Rahilly, Li F. Chan, Adrian J. Clark, I. Sadaf Farooqi and Joseph A. Majzoub

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Accessory to Obesity?

Melanocortin receptors are a family of cell membrane receptors that control diverse physiological functions. Mutations in the gene encoding melanocortin 4 receptor (MC4R) are a cause of familial early-onset obesity. **Asai *et al.*** (p. 275) studied the function of an accessory protein for MC4R signaling, MRAP2, and found that mice genetically deficient in MRAP2 develop severe obesity. Sequencing of *MRAP2* in unrelated, severely obese humans revealed one individual with a clearly disruptive genetic variant, suggesting that *MRAP2* mutations might also be a rare cause of human obesity. In a zebrafish model, **Sebag *et al.*** (p. 278) studied two paralogs of the MRAP2 accessory protein, one of which enhanced MC4R responsiveness to α–melanocyte-stimulating hormone, which regulates feeding and growth.