Loss-of-function mutations in MRAP2 are pathogenic in hyperphagic obesity with hyperglycemia and hypertension

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The G-protein-coupled receptor accessory protein MRAP2 is implicated in energy control in rodents, notably via the melanocortin-4 receptor. Although some MRAP2 mutations have been described in people with obesity1–3, their functional consequences on adiposity remain elusive. Using large-scale sequencing of MRAP2 in 9,418 people, we identified 23 rare heterozygous variants associated with increased obesity risk in both adults and children. Functional assessment of each variant shows that loss-of-function MRAP2 variants are pathogenic for monogenic hyperphagic obesity, hyperglycemia and hypertension. This contrasts with other monogenic forms of obesity characterized by excessive hunger, including melanocortin-4 receptor deficiency, that present with low blood pressure and normal glucose tolerance1. The pleiotropic metabolic effect of loss-of-function mutations in MRAP2 might be due to the failure of different MRAP2-regulated G-protein-coupled receptors in various tissues including pancreatic islets.

According to the World Health Organization, the worldwide prevalence of obesity nearly tripled between 1975 and 2016. About 2 billion people are currently overweight, and their comorbidities represent a major medical burden. The biological link between obesity and type 2 diabetes is still debated, as most individuals with obesity never develop diabetes. If classic views postulate that insulin resistance eventually exhausts insulin-producing cells, alternatively, genetically driven abnormal pathways may lead to both appetite dysregulation and insulin secretion defects, and also to other abnormalities, such as hypertension. While common obesity is seen as a multifactorial disorder, we and others have found that rare mutations in more than 15 genes cause monogenic obesity, including MC4R (encoding the melanocortin-4 receptor) that is the most frequently mutated gene in monogenic obesity4. Importantly, characterization of these mutations has enabled the development of new drugs (such as the MC4R agonist seltmelanotide in patients deficient for POMC or LEPR)5,6. Recently, a study has shown that loss of function of Mrap2 (encoding melanocortin-2 receptor accessory protein 2) is associated with rodent obesity7. The authors demonstrated that MRAP2 interacted directly with MC4R and enhanced MC4R downstream signaling in response to an MC4R agonist, suggesting that MC4R signaling was a mechanism linking Mrap2 loss of function and obesity8. When sequencing MRAP2 in 976 people with obesity and controls, the authors identified four rare variants in four participants with severe obesity that were not present in the controls and suggested MRAP2 as a new gene causing monogenic obesity9. The authors did not perform statistical or functional analyses of these variants. Another rare nonsynonymous variant was described in a patient with obesity associated with Prader–Willi-like syndrome, but still without functional assays10. Furthermore, Schonnop et al. described a rare MRAP2 mutation (encoding p.Q174R) decreasing MC4R activity in vitro in a girl with severe obesity11. Here, we performed a large-scale resequencing study of MRAP2, in combination with functional assays of detected variants, to accurately decipher the functional link between MRAP2 signaling and obesity (and possibly other phenotypes) in humans.

The coding exons of MRAP2 were sequenced in 9,418 participants, including 7,239 adults and 2,179 children or adolescents (Supplementary Table 1). We detected 23 rare heterozygous variants (with a minor allele frequency (MAF) between 0.053‰ and 1.65‰), of which 14 were novel (Table 1 and Fig. 1). The cluster of rare MRAP2 variants was significantly associated with an increased
Table 1 | Rare MRAP2 variants detected in the present study including 1,991 adults with obesity, 2,465 adults with overweight, 2,783 adults with normal weight, 1,137 children or adolescents with obesity, and 1,042 children or adolescents with normal weight

| Chr. | Pos. hg19 | Mutation* | MAF (%) | PVS1 | PS1 | PS2 | PS3 | PM1 | PM2 | PM4 | PM5 | PPI | PP2 | PP3 | Pathogenicity | Number and status of carriers |
|------|-----------|------------|---------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----------------|-------------------------------|
| 6    | 84765033  | c.–5_5del, p.? | 0.053   | 1    | 0   | NA  | NA  | 0   | 1   | 0   | 0   | NA  | 1   | NA  | Pathogenic (LOF) | 1 ob child                   |
| 6    | 84765035  | c.–3_7del, p.? | 0.053   | 1    | 0   | NA  | 1   | LOF | 0   | 1   | 0   | 0   | NA  | 1   | NA  | Pathogenic (LOF) | 1 ob adult                   |
| 6    | 84765044  | c.7G>A, p.A3T  | 0.053   | 0    | 0   | NA  | 0   | 0   | 0   | 0   | NA  | 1   | 0   | VUS |                 | 1 ovw adult                   |
| 6    | 84765044  | c.7G>T, p.A3S  | 0.16    | 0    | 0   | NA  | 0   | 1   | 0   | 0   | NA  | 1   | 0   | VUS |                 | 2 ob children, 1 ob adult    |
| 6    | 84765074  | c.37C>G, p.Q13E | 0.053   | 0    | 0   | NA  | 0   | 0   | 1   | 0   | 0   | NA  | 1   | 0   | VUS |                 | 1 nw child                   |
| 6    | 84765129  | c.92G>T, p.G31V | 0.053   | 0    | 0   | NA  | 1   | LOF | 0   | 1   | 0   | 0   | NA  | 1   | NA  | Lik. pathogenic (LOF) | 1 ob adult                   |
| 6    | 84765132  | c.95C>T, p.P32L | 0.053   | 0    | 0   | NA  | 0   | 0   | 1   | 0   | 0   | NA  | 1   | 1   | VUS |                 | 1 nw child                   |
| 6    | 84772669  | c.185T>G, p.F62C | 0.053   | 0    | 0   | NA  | 1   | LOF | 0   | 1   | 0   | 0   | NA  | 1   | 1   | Lik. pathogenic (LOF) | 1 ovw adult                   |
| 6    | 84798812  | c.230G>T, p.N77S | 0.11    | 0    | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 1   | 0   | Lik. pathogenic (LOF) | 1 ob child, 1 ob adult        |
| 6    | 84798854  | c.272T>C, p.V91A | 0.053   | 0    | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 0   | 1   | 0   | VUS |                 | 1 nw child                   |
| 6    | 84798877  | c.295G>A, p.E99Q | 0.053   | 0    | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 1   | VUS |                 | 1 ob adult                   |
| 6    | 84798886  | c.304A>G, p.K102* | 0.053   | 1    | 0   | NA  | 1   | LOF | 0   | 1   | 0   | 0   | NA  | 1   | NA  | Pathogenic (LOF) | 1 ovw adult                   |
| 6    | 84798919  | c.337A>G, p.R113G | 0.11    | 0    | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 1   | 1   | VUS |                 | 1 ob child, 1 ob adult        |
| 6    | 84798922  | c.340T>G, p.S114A | 0.053   | 0    | 0   | NA  | 0   | 0   | 1   | 0   | 0   | NA  | 1   | 1   | VUS |                 | 1 ob child                   |
| 6    | 84798944  | c.362A>G, p.N121S | 0.053   | 0    | 0   | NA  | 0   | 0   | 1   | 0   | 0   | NA  | 1   | 0   | VUS |                 | 1 ob child                   |
| 6    | 84798955  | c.373C>T, p.R125C | 0.69    | 0    | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 0   | 0   | VUS |                 | 4 ob + 1 nw children, 2 ob + 4 ovw + 2 nw adults |
| 6    | 84798956  | c.374G>A, p.R125H | 1.65    | 0    | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 0   | VUS |                 | 6 ob + 3 nw children, 9 ob + 10 ovw + 3 nw adults |
| 6    | 84798977  | c.397C>T, p.H133Y | 0.053   | 0    | 0   | NA  | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 0   | VUS |                 | 1 nw child                   |
| 6    | 84798991  | c.409G>A, p.A137T | 0.11    | 0    | 0   | NA  | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 0   | VUS |                 | 1 nw child, 1 nw adult       |
| 6    | 84799067  | c.485T>C, p.M162T | 0.11    | 0    | 0   | NA  | 0   | 0   | 1   | 0   | 0   | NA  | 1   | 0   | VUS |                 | 1 ob + 1 nw adult            |
| 6    | 84799159  | c.577A>G, p.T193A | 0.053   | 0    | 0   | NA  | 0   | 0   | 1   | 0   | 0   | 0   | 0   | 1   | VUS |                 | 1 ob adult                   |
| 6    | 84799166  | c.584C>T, p.M195L | 0.16    | 0    | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 1   | 1   | 0   | Lik. pathogenic (LOF) | 1 ob child, 1 ob + 1 owv adult |
| 6    | 84799189  | c.607G>T, p.D203Y | 0.053   | 0    | 0   | NA  | 0   | 0   | 0   | 0   | NA  | 1   | 0   | VUS |                 | 1 nw adult                   |

Chr., chromosome; Lik., likely; LOF, loss of function; NA, not available; nw, normal weight; ob, obese; ovw, overweight; PM1, PM2, PM4 or PM5, ‘moderate’ pathogenicity ACMG criterion; Pos., position (according to the human alignment hg19/GRCh37); PPI, PP2 or PP3, ‘supporting’ pathogenicity ACMG criterion; PS1, PS2 or PS3, ‘strong’ pathogenicity ACMG criterion; PVS1, ‘very strong’ pathogenicity ACMG criterion; VUS, variant of uncertain significance. *All mutations were heterozygous.
The risk of obesity in adults ($r_{variant} = 14; P = 8.04 \times 10^{-4}$ with an odds ratio (OR) of 3.80, 95% confidence interval (CI): 1.71–9.26) and in children or adolescents ($r_{variant} = 13; P = 0.0148$ with an OR of 2.91, 95% CI: 1.23–7.32). When we added the participants with overweight in the adult case–control study, the cluster of rare MRAP2 variants was still significantly associated with an increased risk of adiposity ($r_{variant} = 17; P = 2.25 \times 10^{-3}$ with an OR of 3.13, 95% CI: 1.53–7.27). These data are in line with exome sequencing data from 42,992 participants included in the Accelerating Medicine Partnership Type 2 Diabetes Knowledge Portal, where protein-truncating or missense MRAP2 variants (with MAF < 1% in each ancestry) were significantly associated with increased body mass index (BMI; $r_{variant} = 46; P = 3.49 \times 10^{-4}$ with $\beta = 0.0364\, \text{kg}\, \text{m}^{-2}$, 95% CI: 0.0165–0.0564 kg m$^{-2}$). This association was even stronger when focusing on protein-truncating or probably deleterious missense MRAP2 variants ($r_{variant} = 9; P = 2.36 \times 10^{-2}$ with $\beta = 0.154\, \text{kg}\, \text{m}^{-2}$, 95% CI: 0.0828–0.226 kg m$^{-2}$).

Here, we used the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG) to assess the pathogenicity of each detected variant. To address the strong pathogenic ACMG criterion P3, we analyzed the functional effect of each MRAP2 variant on MC4R activity. MC4R and mutant or wild-type MRAP2 were overexpressed in Chinese hamster ovary (CHO) cells and cyclic (cAMP)-dependent protein kinase (PKA) signaling was analyzed through luciferase reporter assays in response to $\alpha$-melanocyte-stimulating hormone ($\alpha$-MSH; the canonical agonist of MC4R) and adrenocorticotropic hormone (ACTH; another MC4R agonist in the presence of MRAP2; Extended Data Fig. 1)\(^{11-15}\).

When compared to wild-type MRAP2, we found that six MRAP2 variants (c.-3.7del (of unknown effect on the protein), p.G31V, p.F62C, p.N77S, p.K102* and p.P195L) significantly decreased cAMP–PKA signaling downstream of MC4R in response to $\alpha$-MSH and ACTH (Extended Data Fig. 2). These loss-of-function variants were mostly located in highly conserved loci (Fig. 1). According to ACMG criteria (now including the functionally based criterion P3), we identified seven pathogenic or likely pathogenic, loss-of-function variants (c.-5.5del, c.-3.7del, p.G31V, p.F62C, p.N77S, p.K102* and p.P195L; Table 1). These variants were identified in seven adults of European origin with obesity or overweight and in three European adolescents with obesity (Table 2). Therefore, they were completely penetrant for obesity or overweight. Two mutations (encoding p.N77S and p.P195L) co-segregated with obesity in two families that were available for segregation analysis (Extended Data Fig. 3). The majority (75%) of the carriers reported abnormal eating behavior (overeating, snacking and/or bulimia; Table 2). According to the serum levels of 15 steroid hormones (including cortisol, cortisone, testosterone and aldosterone), none of the carriers had dysfunction of the hypothalamic–pituitary–adrenal axis (Supplementary Table 2). We found that all carriers, except for one adolescent (participant 10), presented with the phenotypes of metabolic syndrome (Table 2). Apart from high adiposity, the two most frequent metabolic features were hyperglycemia and hypertension (Table 2 and Fig. 2). This is notable because the prevalence of the aggregation of these phenotypes that constitute so-called metabolic syndrome among people with (polygenic) overweight or obesity usually ranges from 20–60%, according to our current cohort analysis (Supplementary Table 3) and the literature\(^{11}11\). Notably, when compared to other monogenic forms of obesity, including those due to a deficiency of LEP, LEPR, MC4R, PCSK1, POMC or SIM1, MRAP2 deficiency is singular because it leads to a markedly higher rate of hyperglycemia and hypertension (Fig. 2 and Supplementary Table 4). Among the participants who were deficient in MRAP2, we did not find any other (likely) pathogenic variant in 48 genes known to be involved in monogenic obesity or in monogenic diabetes, except for in the 17-year-old participant 10. In this person, we found that all carriers, except for one adolescent (participant 10), presented with the phenotypes of metabolic syndrome (Table 2). Apart from high adiposity, the two most frequent metabolic features were hyperglycemia and hypertension (Table 2 and Fig. 2). This is notable because the prevalence of the aggregation of these phenotypes that constitute so-called metabolic syndrome among people with (polygenic) overweight or obesity usually ranges from 20–60%, according to our current cohort analysis (Supplementary Table 3) and the literature\(^{11}11\). Notably, when compared to other monogenic forms of obesity, including those due to a deficiency of LEP, LEPR, MC4R, PCSK1, POMC or SIM1, MRAP2 deficiency is singular because it leads to a markedly higher rate of hyperglycemia and hypertension (Fig. 2 and Supplementary Table 4). Among the participants who were deficient in MRAP2, we did not find any other (likely) pathogenic variant in 48 genes known to be involved in monogenic obesity or in monogenic diabetes, except for in the 17-year-old participant 10. In this person, we also found a likely pathogenic mutation in ABCG8 (NM_000352.4: c.647G>A, p.R216H). Pathogenic gain-of-function mutations in ABCG8 cause monogenic diabetes in infancy, whereas loss-of-function mutations cause hypoglycemic episodes owing to inappropriate hyperinsulinism\(^{12,13}\). In view of participant 10’s low fasting glucose level (4.33 mM) with a surprisingly elevated fasting insulin level (88.9 pm), it is possible that this presumably inactivating ABCC8 mutation increases insulin secretion, thus normalizing glycemia and masking the metabolic abnormalities caused by the pathogenic MRAP2 mutation.

As the participants who were deficient in MRAP2 mostly presented with hyperglycemia in addition to high adiposity, we assessed MRAP2 expression in a panel of human tissues using a PCR-free technology that we previously described and validated\(^{14}\). We found that MRAP2 expression levels were similarly high in human pancreatic islets and beta cells, the human beta cell line EndoC-J1 and brain regions (Extended Data Fig. 4a). We confirmed MRAP2 protein expres-
sion in human islets and EndoC-β1 cells by western blot analysis (Extended Data Fig. 4b). On the basis of these data, we assessed the involvement of MRAP2 in beta cell function by performing a glucose-stimulated insulin secretion assay in EndoC-β1 cells15, in which MRAP2 expression was decreased with a specific siRNA. We found that the fold change in the insulin concentration was significantly increased.

### Table 2 | Clinical data of participants carrying a pathogenic loss-of-function MRAP2 variant

| Participants | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|--------------|---|---|---|---|---|---|---|---|---|---|
| MRAP2 mutation | c.-3_7del | p.G31V | p.F62C | p.N77S | p.K102* | p.P195L | p.P195L | c.-5_5del | p.N77S | p.P195L |
| Ancestry | Eur | Eur | Eur | Eur | Eur | Eur | Eur | Eur | Eur | Eur |
| Age at investigation (years) | 44 | 48 | 44 | 43 | 61 | 61 | 49 | 13 | 12 | 17 |
| Sex (M/F) | F | F | M | M | F | F | F | M | F |
| BMI | 49.3 | 30.1 | 25.3 | 49.6 | 25.3 | 32.81 | 279 | 34.8 | 30.9 | 29.7 |
| BMI for age (percentile) | - | - | - | - | - | - | - | 99th | 99th | 95th |
| Obesity | Class III | Class I | Overweight | Class III | Overweight | Class I | Overweight | Severe obesity | Severe obesity | Obesity |
| Age of obesity onset (years) | - | - | - | - | - | - | - | - | 2 | 8 |
| Obesity during childhood or adolescence (yes/no) | Yes | - | - | Yes | - | - | - | Yes | Yes | Yes |
| Eating behavior | Continuous diet | None | Snacking | Continuous diet | None | - | Bulimia and snacking | - | Overeating and snacking | Overeating and bulimia |
| Treatment | Antidepressant | AHT | None | None | Cholesterol-lowering drug | AHT/cholesterol-lowering drug | None | - | None | None |
| Fasting glucose (mM) | 7.21 | 6.14 | 6.05 | 5.50 | 5.77 | 6.16 | 6.59 | 5.30 | 5.70 | 4.33 |
| 2h glucose during an OGTT (mM) | 11.4 | - | - | 5.70 | - | 12.5 | - | - | 7.20 | 5.66 |
| Fasting insulin (pM) | 122 | 149 | 35.9 | 25.0 | 57.7 | 97.2 | 74.5 | 37.0 | 181 | 88.9 |
| HOMA2-%B | 83.0 | 129 | 49.6 | 38.8 | 75.4 | 95.1 | 69.4 | 66.0 | 170 | 178 |
| HOMA2-IR | 2.44 | 2.85 | 0.710 | 0.490 | 1.12 | 1.89 | 1.48 | 0.710 | 3.38 | 1.58 |
| Waist-to-hip ratio | 1.01 | 0.902 | 0.918 | 0.947 | 0.960 | - | 0.852 | - | 1.06 | 0.866 |
| Waist circumference (cm) | 131 | 101 | 90 | 142 | 95 | - | 92 | - | 103 | 103 |
| SBP (mm Hg) | - | 140 | 138 | 160 | 143 | 150 | 128 | - | 120 | - |
| DBP (mm Hg) | - | 85 | 80 | 100 | 93 | 90 | 80 | - | 80 | - |
| TC (mM) | 5.28 | 6.66 | 7.07 | 3.66 | 5.40 | 9.00 | 6.53 | - | 5.06 | 4.62 |
| HDL (mM) | 0.820 | 1.19 | 1.21 | 1.08 | 1.63 | 1.44 | 1.83 | - | 1.08 | 1.39 |
| TG (mM) | 1.19 | 1.62 | 0.980 | 1.77 | 1.00 | 4.67 | 1.72 | - | 2.27 | 0.900 |
| Hypertension (yes/no) | - | Yes | Yes | Yes | Yes | Yes | No | - | No | - |
| Diabetes | T2D | PD | PD | NGT | PD | T2D | PD | NGT | PD | NGT |
| Metabolic syndrome (yes/no) | Yes | Yes | Yes | Yes | Yes | Yes | Yes | - | Yes | No |

AHT, antihypertensive drug; DBP, diastolic blood pressure; Eur, European; F, female; HOMA2-%B, homeostasis model assessment of steady-state beta cell function; HOMA2-IR, homeostasis model assessment of insulin resistance; M, male; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PD, prediabetes; SBP, systolic blood pressure; T2D, type 2 diabetes; TC, total cholesterol; TG, triglycerides.
loss-of-function metabolic phenotypes observed in people carrying a pathogenic, obesity with appetite dysfunction described so far, including MC4R is phenotypically very different from other forms of monogenic obesity due to pathogenic loss-of-function mutations in could have a direct functional deleterious effect on beta cells. Might be treated by the MC4R agonist setmelanotide7. MRAP2 in. Because MRAP2 deficiency partly impacts the receptors across various key tissues, explaining the pleiotropic effect of MRAP2 on metabolically active G-protein-coupled insulin levels and normal tolerance to intraperitoneal glucose injection1. Two subsequent studies reported that Mrap2-null mice had normal fasting insulin levels and normal tolerance to intraperitoneal glucose injection1, two subsequent studies reported that Mrap2-null mice (with different genetic backgrounds from the previously tested Mrap2-null mice) had impaired glucose homeostasis under fasting or after a glucose or insulin tolerance test14,15. Rouault et al. also demonstrated that impaired glucose tolerance of Mrap2 knockout mice occurred before they developed obesity16. These results suggest that MRAP2 mutations could have a direct functional deleterious effect on beta cells.

In conclusion, we describe a monogenic form of hyperphagic obesity due to pathogenic loss-of-function mutations in Mrap2, which is also associated with hyperglycemia and hypertension. This is phenotypically very different from other forms of monogenic obesity with appetite dysfunction described so far, including MC4R deficiency, where patients with obesity along with high plasma glucose levels and high blood pressure have been infrequently reported (Fig. 2 and Supplementary Table 4)1. In addition to brain and pancreatic islets, MRAP2 is expressed in several human metabolic tissues, including in the gut, kidney, adipose tissue and skeletal muscle (Extended Data Fig. 4a). Mrap2 not only regulates the activity of melanocortin receptors but is also involved in the regulation of other G-protein-coupled receptors including prokineticin receptors and the growth hormone secretagogue receptor 1a (GHSR1a), namely the ghrelin receptor17,18. Therefore, we suggest that the spectrum of metabolic phenotypes observed in people carrying a pathogenic, loss-of-function Mrap2 mutation is due to the impaired regulatory effect of MRAp2 on metabolically active G-protein-coupled receptors across various key tissues, explaining the pleiotropic effect of the encoded protein. In this regard, ghrelin and its receptor have been involved in blood pressure regulation15, which could explain the high blood pressure observed in the individuals deficient for MRAp2. Because MRAp2 deficiency partly impacts the MC4R pathway, the eating behavior problems in people deficient in MRAp2 might be treated by the MC4R agonist setmelanotide7.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information, details of author contributions and competing interests, and statements of data and code availability are available at https://doi.org/10.1038/s41591-019-0622-0.

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Fig. 2 | Rates of hyperglycemia, hypertension, low HDL and high TG in patients deficient for LEP, LEPR, MC4R, PCSK1, POMC, SIM1 or MRAP2. Orange areas show rates of hyperglycemia, hypertension, low HDL and high TG, whereas blue areas show rates of normal glucose, normal blood pressure, high HDL and low TG. This figure was generated using the data in Table 2 (for MRAp2 deficiency) and Supplementary Table 4 (for deficiencies of LEP, LEPR, MC4R, PCSK1, POMC and SIM1). HDL, high-density lipoprotein; NA, not available; TG, triglycerides.
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Methods

Study participants. We investigated 9,418 blood DNA samples from several population studies: (1) 3,766 participants from the DESIR (Data from the Epidemiological Study on the Insulin Resistance Syndrome) 9-year prospective study, including middle-aged men and women from western France; (2) 13,394 participants who were recruited and followed by the CNRS UMR8199 (Lille, France), by the Department of Nutrition of Hotel-Dieu Hospital (Paris, France) or by the Centre d’Étude du Polyomorphisme Humain (Saint-Louis Hospital)31,32; (3) 929 participants who were recruited from the Department of Endocrinology of the Corbeil-Essonnes Hospital (Corbeil-Essonnes, France); (4) 672 participants from the French Haguenau regional cohort study32; and (5) 457 participants from the French Fleurbaix-Laventie Ville Santé study33. Clinical data of participants are shown in Supplementary Table 1. The study protocols were approved by local ethics committees. All participants older than 18 years signed an informed consent form. Oral assent from children or adolescents was obtained and parents (or legal guardians) signed an informed consent form.

In participants older than 18 years, class I obesity was defined as BMI ≥ 30 kg/m2, class II as BMI ≥ 35 kg/m2, class III as BMI ≥ 40 kg/m2, overweight as BMI ≥ 25 kg/m2 and normal weight as BMI < 25 kg/m2. In children and adolescents younger than 18 years, obesity was defined as 95th percentile and severe obesity was defined as BMI for age ≥ 99th percentile, whereas normal weight was defined as BMI for age < 85th percentile, according to the US Centers for Disease Control and Prevention growth charts.

Type 2 diabetes was defined as fasting plasma glucose ≥ 7.0 mM, plasma glucose measured at 2 h during an OGTT ≥ 11.1 mM and/or hyperglycemia treatment; hyperglycemia or prediabetes was defined as fasting plasma glucose ≥ 5.6 mM or < 7.0 mM without hyperglycemia treatment and normal glucose was defined as fasting plasma glucose < 5.6 mM without hyperglycemia treatment25.

Metabolic syndrome was defined following the criteria of the third report of the National Cholesterol Education Program expert panel on detection, evaluation and treatment of high blood cholesterol in adults (National Cholesterol Education Program). A waist circumference > 94 cm in men and > 80 cm in women was used as an additional criterion for the diagnosis of metabolic syndrome.

For NGS, target enrichment was performed according to the manufacturer’s protocol (NimbleGen SeqCap EZ) for Illumina sequencing. Briefly, 1 ng of DNA was fragmented by Covaris E220 Focused-ultrasonicator. The fragmented DNA samples were end-repaired and ligated to the adaptors using KAPA Library Preparation kits (Boston, Massachusetts) with adapter ligation (KAPA) and a single-pool approach. These samples were subsequently amplified by PCR. After size selection and sample quantification (PerkinElmer LabChip GX), 24 samples were combined in a single pool of at least 1 µg and hybridized to the biotin-labeled SeqCap EZ probe pool. After 72 h at 47 °C, the cultures were purified using the SeqCap Hybridization and Wash kit on the Agilent Bravo Automated Liquid Handling Platform. Captures were subsequently amplified using KAPA HiFi HotStart ReadyMix and quantified by using Infecterin LabChip GX and Thermo Fisher Scientific Qubit fluorometric quantification assays. Then, the samples were sequenced on the Illumina HiSeq 4000 system (with a throughput of one pool per lane), using a paired-end 2 × 150-bp protocol. Demultiplexing of sequence data was performed using bcftools Conversion Software (Illumina; v.2.17). Subsequently, sequence reads were mapped to the human genome (hg19/GRCh37) using Burrows–Wheeler Aligner (v.0.7.13)34. Variant calling was performed using the Genome Analysis Toolkit (GATK; v.3.3)30. Only variants with a coverage of greater than eight reads were kept for further analyses. Annotation of variants was performed using Ensembl Perl Application Program Interfaces (v.73) and custom Perl scripts to include data from both the dbSNP (v.135) and dbNSFP (v.3.0) databases35–37. All detected variants in MRAP2 had a QAL score ≥ 50, and therefore, no variant was found with coverage below eight reads or a QAL score < 50 across the participants, and no participant had < 5% missing genotypes (with coverage below eight reads or a QAL score < 50) across MRAP2.

The location of each variant (detected either by Sanger sequencing or NGS) was determined using the translation initiation codon of each gene in the International Human Genome Variation Society nomenclature for the description of sequence variations. The positions of variations are indicated according to human genome build hg19/GRCh37.

Sequencing of genes involved in monogenic obesity or monogenic diabetes. In the ten carriers of a pathogenic loss-of-function MRAP2 mutation, we analyzed the presence of a pathogenic or likely pathogenic variant in 48 known genes involved in monogenic obesity or monogenic diabetes via NGS: ABCG8, ADCA3, ADcy3, APPL1, BDFN, BLK, CEL, CEPI9, DNAJC13, Dyrk1B, Foxp3, GATA4, GCK, GLIS3, GNAS, HNF1A, HNF1B, HNF4A, IER3IIN1, INS, KCNJ11, KLF11, KR2, LEP, LEPR, MAGEL2, MC4R, MNNX1, NEUROD1, NEUROG3, NKX2-2, NTRK2, PAX4, PAX6, PCD1, PCSK1, PDX1, PMC, PTF1A, RFX6, SH2B1, SLC19A2, SLC2A2, STAT3, TRMT10A, TUB and WFS1.

Variant sequence analysis. The ancestry of individuals with mutations was assessed using the first two genotypic principal components (PC1 and PC2) calculated from at least 10,000 single-nucleotide polymorphisms (with MAF > 5%) present in each of these individuals (where genotypes were obtained from DNA arrays or NGS data) and in the 1000 Genomes Project.

Design of variant analysis. We only analyzed rare variants of potential interest (missense variants, stop-gained, frameshift variants, in-frame variants, initiator-codon variants, stop-retained variants, stop-lost variants, splice-donor variants and splice-acceptor variants) with MAF < 1%. We assessed the pathogenicity of the variants using the ACMG criteria for the interpretation of rare variants (Supplementary Tables 5 and 6)30. To address the strong pathogenic criterion PS3, we used our in-house in vitro functional analyses (see below).

In vitro functional analyses. Twenty-two variants (including 1 frameshift variant and 21 missense variants) detected in the participants and 1 negative-control variant (c.10C>T, p.Q4>*) were introduced in 23 different plasmids (pcDNA3.1 MRAP2) using the QuikChange site-directed mutagenesis kit (Stratagene). Each novel plasmid was checked by Sanger sequencing. The effect of each MRAP2 variant on MC4R activity was assessed in CHO cells in response to different concentrations (0–30 nM) of α-MSH and ACTH. For this purpose, CHO cells were cultured in DMEM F12 with 10% FBS and 1% penicillin-streptomycin (Gibco) at 37 °C and 5% CO2. Cells were transfected with 225 ng ml−1 plasmid encoding the firefly luciferase gene under the control of a CAMP response element, 150 ng ml−1 plasmid encoding the β-galactosidase gene, 41.25 ng ml−1 MC4R plasmid and either 333.75 ng ml−1 wild-type MRAP2 plasmid or 333.75 ng ml−1 mutated MRAP2 plasmid using FuGENE 6 (Promega) and were seeded in 48-well plates at a concentration of 600,000 cells per ml in a volume of 200 µl. The day after, the medium was replaced by medium with 0.1% FBS overnight serum starvation; 48 h after transfection, the cells were treated with increasing doses of OCA (Abnormal or ACTH (Sigma) from 0 to 30,000 µM. At 5 h after treatment, the cells were lysed with 100 µl of luciferase cell culture lysis buffer (Promega). The luminescence was assessed by adding 25 µl of the luciferase assay system reagent (Promega) to 40 µl of lysate and was read using a Glomax luminometer (Promega). β-galactosidase activity was measured after incubation of 40 µl of lysate for 5 min with 100 µl of homemade buffer (0.477 g NaHPO4, 1.060 g Na2HPO4, 2H2O, 0.5 ml KCl, 2 M, 0.1 M l-MgCl2, q.s. to 100 ml of water; extemporaneous addition of β-mercaptoethanol (325 µl) and 4 mM ortho-nitrophenyl-β-galactoside (25 mM)) and was read at 450 nm. Of note, we found that the best profile of MC4R activation in response to α-MSH was obtained using 1 volume of MC4R plasmid in addition to 8 volumes of MRAP2 plasmid, when compared to 1:2 and 1:1 MC4R:MRAP2 ratios (data not shown). The experiments were performed in technical triplicate and each experiment was repeated three times. Luciferase measurements in relative luminescence units were normalized using β-galactosidase measures. Fold change was computed by dividing the normalized luciferase activity (L∞) by the mean of the baseline luciferase activity. This normalized fold change in luciferase activity (FC∞) was analyzed using a linear regression model. The mutation (M); the agonist concentration (C), represented as an orthogonal polynomial function of degree 3 (PC3) to enable possible nonlinear relationships between FC∞ and C; and the interaction term (PM) between M and P∞ were included in the model as covariates.

The model was defined as follows:

\[
FC∞ = βΓ + βΓM + MP∞ + P∞ + ε
\]

with

\[
P∞ = αΓC2 + αΓC + αC2M
\]

\[
MP∞ = θ1C2M + θ2C2M + θ3C4M
\]

Our functional assay was validated using a negative-control variant (p.Q4*; Supplementary Fig. 2).

MRAP2 expression analysis. Expression analysis of MRAP2 was performed using NanoString technology, which is multiplex digital quantification of nucleic acids (vs. conventional PCR with a biased amplification), in a large panel of human tissues, as previously described14. The panel included human RNA from the colon, small intestine, liver, kidney, adipose tissue, placenta, lung, skeletal muscle, heart, brain, substantia
nigra, hippocampus, dorsal root ganglion, insula, hypothalamus, pituitary gland, caudate nucleus, frontal lobe, pancreatic islets, pancreatic beta cells (obtained by laser capture microdissection or sorted by flow cytometry), exocrine pancreas and the pancreatic beta cell line EndoC-βH1.

Western blot analysis. Proteins were extracted from CHO cells transfected or not with 450 ng of MRAP2 plasmid, human pancreatic islets (pool from two donors) and EndoC-βH1 cells, using Pierce RIPA buffer (Thermo Scientific) according to the manufacturer's instructions. Proteins were quantified by the Bradford technique using the Bio-Rad Protein Assay (Bio-Rad). Then, 50 µg (for islets and EndoC-βH1 cells) or 10 µg (for CHO cells) of protein with 4x Laemmli buffer (Alfa Aesar) was denaturated at 95°C for 5 min and loaded for migration on a concentration gel (4% acrylamid (Dutsch)), 125 mM Tris (Dutsch), 0.04% SDS (Sigma), 0.08% ammonium persulfate (Sigma), 0.004% TEMED (Dutsch)) and runned with Tris, 0.2% SDS, 0.1% ammonium persulfate, 0.05% TEMED). Migration was performed in Tris–glycine–SDS buffer (Eurobio-Ingen). After migration, proteins were transferred to a nitrocellulose membrane (Bio-Rad) in Tris–glycine buffer (Eurobio-Ingen). Nonspecific sites were blocked with a 1-h incubation in TBS buffer + 0.1% Tween 20 (BioRad) in T25 flasks coated with β-coat (Univercell) according to the manufacturer's instructions. Cells (500,000 cells per ml) were transfected with either an ON-TARGETplus nontargeting pool (siNTCP) for controls or siRNA targeting MRAP2 (siMRAP2, 20 µM; Horizon Discovery) using Lipofectamine 3000 (Invitrogen) in OptiMEM (Gibco) complemented with 50 µM 2-mercaptoethanol, 10 mM nicotinamide (Calbiochem, Merck Millipore), 5.5 mg ml⁻¹ human transferrin (Sigma-Aldrich), 6.7 ng ml⁻¹ sodium selenite (Sigma-Aldrich), 100 U ml⁻¹ penicillin and 100 mg ml⁻¹ streptomycin (Life Technologies) and seeded (50,000 cells per well) in a 96-well plate coated with (1 mg ml⁻¹) of the cluster.

MRAP2 knockdown and insulin secretion assays in EndoC-βH1 cells. EndoC-βH1 cells were cultured at 37°C in 5% CO₂ with OptiMEM medium (Univercell) in T25 flasks coated with β-coat (Univercell) according to the manufacturer's instructions. Cells (500,000 cells per ml) were transfected with either an ON-TARGETplus nontargeting pool (siNTCP) for controls or siRNA targeting MRAP2 (siMRAP2, 20 µM; Horizon Discovery) using Lipofectamine 3000 (Invitrogen) in OptiMEM (Gibco) complemented with 50 µM 2-mercaptoethanol, 10 mM nicotinamide (Calbiochem, Merck Millipore), 5.5 mg ml⁻¹ human transferrin (Sigma-Aldrich), 6.7 ng ml⁻¹ sodium selenite (Sigma-Aldrich), 100 U ml⁻¹ penicillin and 100 mg ml⁻¹ streptomycin (Life Technologies) and seeded (50,000 cells per well) in a 96-well plate coated with β-coat. After 6 h, the medium was replaced by OptiMEM medium. The day after, the medium was renewed, and 2 d after transfection the medium was replaced by DMEM without glucose (Gibco) supplemented with 2% BSA fraction (Dutsch) under agitation. Then, the membrane was incubated overnight at 4°C under agitation with anti-MRAP2 antibody (Bios; 1:300 dilution in TBS buffer + 0.1% Tween-20 + 5% BSA (Sigma-Aldrich)). After incubation, the membrane was washed three times in TBS buffer + 0.1% Tween-20 and then incubated for 1 h at room temperature under agitation with HRP-conjugated anti-rabbit antibody (Cell Signaling: 1:2,500 dilution in TBS buffer + 0.1% Tween-20 + 5% skim milk). After the incubation, the membrane was washed three times in TBS buffer + 0.1% Tween-20. ECL Prime (Amersham) was used to visualize the protein of interest and reading was performed using a Chemidoc (Bio-Rad) after 120 s of exposure.

The equation for the model is as follows: \( Y = \alpha x + xG \), where \( Y \) is the phenotype matrix (\( n \times 1 \)) for \( n \) individuals, \( \alpha \) is the matrix of covariates (\( n \times p \)) with \( p \) covariates, and \( G \) is a vector of \( q \) ones for the \( q \) variants and \( G \) is the genotype matrix (\( n \times G \)) coded 0, 1 and 2 for AA, Aa, aa, where A is a major allele and a is a minor allele.

As none of the association studies had significant heterogeneity (\( P > 0.25 \)), we only show the \( P \) value associated with the mean effect (\( \tau \)) of the cluster.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
All relevant data have been included in the manuscript and/or in its supplementary tables and figures. Source data are available online for Extended Data Figs. 1, 2 and 5. Targeted DNA-seq data of patients deficient in MRAP2 were deposited in the NCBI Sequence Read Archive under PRJNA564478.

Code availability
Code to perform analyses in this manuscript are available from the authors upon reasonable request (A.B., M.D. and M.C.).

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Author contributions
P.F. and A.B. conceived the idea for the study and supervised the analyses. M.B., J.M., M.H., A.D., R.B., H.L., E.D., B.T., E.V., J.P., J.T., A.G., M.B., M.D., S.G., M.C. and A.B. performed the experiments and/or analyses. M.B. and A.B. wrote the first draft of the paper. P.F. revised the paper. S.E., G.C., J.-M.B., C.L.-M., M.T., R.S., J.W., C.A., J.K.-C., F.P., R.B., B.B., M.M. and P.F. contributed data (cohort studies or beta cell models). Furthermore, all authors critically reviewed the paper and approved the report for submission.

Competing interests
The authors declare no competing interests.

Additional information
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Extended Data Fig. 1 | Design of the functional assessment of each MRAP2 mutation and its validation. α-MSH, α-melanocyte-stimulating hormone; ACTH, adrenocorticotropic hormone; βGal, β-galactosidase; CHO, Chinese hamster ovary cells; pβGal, plasmid including the β-galactosidase gene; pCreLuc, plasmid including the firefly luciferase (Luc) gene under the control of cAMP response element (CRE); pMC4R, plasmid including MC4R; pMRAP2, plasmid including MRAP2; WT, wild-type. For the control of pMRAP2 transfection into CHO cells, we performed Western blot analyses in wild-type CHO cells and CHO cells transfected with pMRAP2. We confirmed that wild-type CHO cells did not endogenously express MRAP2 protein, whereas CHO cells transfected with pMRAP2 expressed MRAP2 protein (27 kDa, bottom asterisk), as well as its glycosylated form (29 kDa, top asterisk). For the negative control, data are cAMP reporter activity (CRE-Luc normalized with β-galactosidase), expressed as fold change after 0–30,000 pM α-MSH (left) or ACTH (right), relative to 0 pM. Data are the mean ± sem of three independent experiments in technical triplicates. Fold change was computed by dividing normalized luciferase (L*) by the mean of the baseline luciferase measures. This normalized luciferase fold change (FCL*) was analyzed using a linear regression model. The mutation (M), the agonist concentration (C), as an orthogonal polynomial function of degree 3 (PC) to enable possible nonlinear relations between FCL and C and the interaction term (MPC) between M and PC were included in the model as covariates. The model was defined as follows: $FCL^* = \beta_0 + \beta_1 M + MP_C + \epsilon$ with, $P_C = \alpha_1 C^1 + \alpha_2 C^2 + \alpha_3 C^3$ $MP_C = \theta_1 C^1 M + \theta_2 C^2 M + \theta_3 C^3 M$ ***$P < 0.001$, MC4R + p.Q4* MRAP2 (red) versus MC4R + wild-type MRAP2 (black).
Extended Data Fig. 2 | Effect of MRAP2 variants on MC4R activity in response to α-MSH and ACTH in CHO cells. Data are cAMP reporter activity (CRE-Luc normalized with β-galactosidase) in CHO cells cotransfected with MC4R plasmid and wild-type or mutated MRAP2 plasmid, expressed as fold change after 0–30,000 pM α-MSH or ACTH, relative to 0 pM. Data are the mean ± sem of three independent experiments in technical triplicates. Fold change was computed by dividing normalized luciferase (L*) by the mean of the baseline luciferase measures. This normalized luciferase fold change (FC*) was analyzed using a linear regression model. The mutation (M), the agonist concentration (C), as an orthogonal polynomial function of degree 3 (PC) to enable possible nonlinear relations between FC* and C, and the interaction term (MPC) between M and PC were included in the model as covariates. The model was defined as follows: 

\[ FC^* = \beta_0 + \beta_1 M + \beta_2 C + \beta_3 PC + \epsilon \]

with,

\[ PC = \alpha_1 C^3 + \alpha_2 C^2 + \alpha_3 C \]

\[ MPC = \theta_1 C^3 M + \theta_2 C^2 M + \theta_3 C^2 M \]

\*P < 0.05, **P < 0.01, ***P < 0.001, MC4R + mutated MRAP2 (colors) versus MC4R + wild-type MRAP2 (black).
Extended Data Fig. 3 | Co-segregation of p.N77S (carried by participants no. 4 and 9) and p.P195L (carried by participants no. 6 and 10) with obesity in two families. HDL, high-density lipoprotein; HT, hypertension; MS, metabolic syndrome; strikethrough MS, no metabolic syndrome; NBP, normal blood pressure; NG, normal fasting glucose; NM, mutation carrier; NN, wild type; NW, normal weight; Ob, obese; PD, prediabetes; SOb, severely obese; TG, triglycerides.
Extended Data Fig. 4 | Expression of MRAP2 in human pancreatic islets and beta cells. **a**, PCR-free quantification of MRAP2 mRNA levels in a panel of human tissues. **b**, Western blot analyses of human islets and EndoC-βH1 cells using MRAP2 antibody. FACS-sorted beta cell, pancreatic beta cells sorted by flow cytometry; LCM beta cell, pancreatic beta cells obtained by laser capture microdissection. Three independent experiments showed similar results for Extended Data Fig. 4b.
Extended Data Fig. 5 | Impaired insulin secretion from EndoC-βH1 cells treated with siRNA targeting MRAP2. EndoC-βH1 cells were transfected with control nontargeting pool siRNA (siNTP) or MRAP2 siRNA (siMRAP2) and were analyzed 72 h thereafter. Insulin secretion (percentage of secretion of the total insulin content) was analyzed in response to 60 min of incubation with 0.5 mM glucose, followed by 60 min of incubation with 16.7 mM glucose. Data are box and whisker plots (with the minimum and the maximum) of four independent experiments (Left). Fold change data are mean values ± sem of four independent experiments (right). Fold change of insulin secretion for siMRAP2 was analyzed using a linear regression adjusted for experimental conditions (operator and date). Glc, glucose.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- Sonolab E-Series (version 6.2) to analyse DNA sonication through the Covaris E220 system, Perkin Elmer LabChip GX software (version 1) to analyse the quality of libraries, Agilent Bravo Automated Liquid Handling Platform software (version 1; for SeqCap EZ capture), Hamilton run control software (version 1; for SeqCap EZ capture), Thermo Fisher Scientific Qubit software (APP version 1.02) to quantify libraries, Illumina HCS software (version 3.4.0) for HiSeq 4000 system, 4th dimension (4D; version 6.7) software to collect patient phenotypes, Microsoft Excel (version 2016)

Data analysis

- bcl2fastq Conversion Software (Illumina; version 2.17), Burrows-Wheeler Aligner (version 0.7.13), Genome Analysis ToolKit (GATK; version 3.3), Ensembl Perl Application Program Interfaces (version 75), custom Perl scripts to include data from both dbSNP (version 135) and dbNSFP (version 3.0) databases, R (version 3.4.2), GraphPad Prism (version 7.04), Image Lab Software (BioRad), Variant Reporter (version 2)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All relevant data have been included in the manuscript and/or in its supplementary tables and figures. Targeted DNA-seq data of MRAP2-deficient patients have been deposited in the NCBI Sequence Read Archive (SRA) under PRJNA564478.
Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**

9,418 participants including 1,991 obese adults, 2,465 overweight adults, 2,783 normal-weight adults, and a young-age case-control study for obesity including 1,137 obese cases and 1,042 normal-weight controls.

**Data exclusions**

Participants with poor sequencing data (following all QCs described in the manuscript). This exclusion criteria was previously established, according to the established QCs.

**Replication**

Appropriate procedures were taken in all experiments to ensure proper sample collection, experimental design, and analysis. We detected the same mutations in samples which were sequenced at least two times through next-generation sequencing (from different library preparations). Each rare MRAP2 variant detected through next-generation sequencing were replicated through Sanger Sequencing. The associations between 1/ increased body mass index and protein-truncating or missense MRAP2 variants, and 2/ increased body mass index and on protein-truncating or probably deleterious missense MRAP2 variants, were replicated in 42,992 participants included in the Accelerating Medicine Partnership (AMP) Type 2 Diabetes knowledge portal.

**Randomization**

All genetic experiments and analyses were performed after randomly allocating cases and controls.

**Blinding**

All genetic experiments and analyses were performed blindly to case-control status as all data and samples were anonymized.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology         |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data         |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

### Antibodies

**Antibodies used**

MRAP2 antibody (Bioss; catalog #bs-11419R; polyclonal; lot #AC060901; dilution: 1/300 in TBS Buffer + 0.1% Tween 20 + 5% Bovine Serum Albumin [Sigma Aldrich]); anti-rabbit HRP antibody (Cell signaling; catalog #7074; polyclonal; dilution: 1/2500 in TBS Buffer + 0.1% Tween 20 + 5% skimmed milk).

**Validation**

MRAP2 antibody was validated using wild-type Chinese hamster ovary (CHO) cells (that do not endogenously express MRAP2) and CHO cells transfected with the human MRAP2 plasmid (Supplementary Figure 1). The two antibodies used were commercial antibodies with validation procedures described on the sites of the companies (ie. Bioss for MRAP2 antibody and Cell signaling for the secondary HRP antibody).

### Eukaryotic cell lines

**Policy information about** [cell lines](#)

**Cell line source(s)**

Chinese hamster ovary (CHO) cells were purchased from ATCC (CHO-K1; ATCC Catalog No. CCL-61); EndoCbetaH1 cells were purchased from Endocells.

**Authentication**

The cells used in this study were not authenticated.
**Human research participants**

**Policy information about studies involving human research participants**

### Population characteristics

| Age Group                          | BMI Definitions                              | Number of Participants | Mean Age (±SD) | Mean BMI (±SD) | Mean Fasting Glucose (±SD) |
|------------------------------------|----------------------------------------------|------------------------|----------------|----------------|---------------------------|
| Overweight adults                  | 25 ≤ BMI < 30 kg/m²                          | 2,465                  | 52.4 ± 12.0   | 27.2 ± 1.39    | 6.16 ± 1.99               |
| Normal weight adults               | BMI < 25 kg/m²                               | 1,042                  | 17.5 ± 3.30   | 20.2 ± 2.32    |                           |
| Obese adults                       | 30 ≤ BMI < 35 kg/m²                          | 1,991                  | 49.0 ± 12.7   | 40.2 ± 8.76    | 6.48 ± 2.43               |
| Severe obesity adults              | BMI ≥ 40 kg/m²                               |                        |                |                |                           |
| Overweight adolescents             | 25 ≤ BMI < 30 kg/m²                          | 2,783                  | 47.2 ± 11.7   | 22.3 ± 1.83    | 5.49 ± 1.42               |
| Normal weight adolescents          | BMI < 25 kg/m²                               | 1,137                  | 12.3 ± 2.35   | 17.0 ± 5.56    |                           |

**Recruitment**

1/ 3,766 participants from the D.E.S.I.R. 9-year prospective study including middle-aged men and women from western France.
2/ 3,594 participants who were recruited and followed-up either by the CNRS UMR8199 (Lille, France), by the Department of Nutrition of Hotel-Dieu Hospital (Paris, France), or by the Centre d’Etude du Polymorphisme Humain (CEPH, Saint-Louis Hospital, Paris, France).
3/ 929 participants who were recruited from the Department of Endocrinology of the Corbeil-Essonnes Hospital (Corbeil-Essonnes, France).
4/ 672 participants from the French Haguenau regional cohort study.
5/ 457 participants from the French Fleurbaix-Laventie Ville Santé study.

Study enrollment in these populations was voluntary. Therefore, biases of this study are similar to those of any biobank with voluntary enrollment. In particular, individuals included in prospective studies like D.E.S.I.R. are usually taking better care of their health, compared to more general populations. Therefore, they tend to be "super controls", which might have increased the statistical power. Furthermore, the majority of cases were enrolled in hospitals. Therefore, compared to patients from primary care, the severity of their disease might be higher. However, here, none of MRAP2-deficient individuals was recruited from specialized department of endocrinology in hospitals.

**Ethics oversight**

All cohort studies followed ethical principles defined in the Helsinki declaration, and they were approved by local ethical committees from Corbeil-Essonnes hospital (France), Comité Consultatif de Protection des Personnes se prêtant à des Recherches Biomédicales (CPPRB) of Lille - Lille hospital (Lille, France), Hotel-Dieu hospital (France), Bicêtre hospital (France).

Note that full information on the approval of the study protocol must also be provided in the manuscript.