

A frameshift mutation in *MC4R* associated with dominantly inherited human obesity

The melanocortin-4 receptor (*MC4R*) is a G-protein coupled, seven-transmembrane receptor which is highly expressed in the hypothalamus, a region of the brain intimately involved in appetite regulation¹. It is a high-affinity receptor for α MSH, a product of the pro-opiomelanocortin (*POMC*) gene, which inhibits feeding when administered to rodents². Hypothalamic *POMC* neurons are stimulated by leptin, an adipocyte-specific hormone which regulates appetite and energy expenditure, and constitute a link between leptin and the melanocortin system. *Mc4r*-deficient mice are hyperphagic, severely obese, hyperinsulinaemic and show increased linear growth³. Mice heterozygous for a null *Mc4r* allele exhibit weight gain intermediate to that seen in wild-type and homozygous mutant littermates. Additionally, ectopic expression in the brain of agouti⁴ and agouti-related transcript⁵, natural antagonists of the *MC4R* ligand, α MSH, results in obesity in rodents. In humans, obesity syndromes associated with abnormalities in *POMC* (ref. 6) and prohormone processing defects involving *POMC* (ref. 7) have also been described.

We have identified a cohort of severely obese children in whom no evidence for a recognized clinical syndrome or a structural hypothalamic cause for their obesity has been found. All are severely obese (mean body mass index (weight/height²) is 34 kg/m²) from an early age (<10 years). Sixty-three of these subjects were screened

for mutations in *MC4R* by direct nucleotide sequencing. We identified one subject who was heterozygous for a 4-bp deletion at codon 211 (Fig. 1b). This results in a frameshift that introduces five aberrant amino acids culminating in a stop codon in the region encoding the fifth transmembrane domain, resulting in a truncated protein. As residues at the base of the fifth and sixth transmembrane domains are needed for G-protein binding and activation⁸, this mutation is likely to result in a non-functional receptor. No mutations were found in the 62 other subjects studied.

The index patient II.1 (Fig. 1a) is four years old and is the only child from a non-consanguineous union. His weight is 32 kg (>99th centile), height 107 cm (91st centile) and body mass index (BMI) is 28 kg/m² (>99th centile). His birthweight was 3.8 kg (50th centile), and progressive weight gain was noted from the age of four months (Fig. 2a). There is no clinical or biochemical evidence of adrenal or thyroid disease, the subject has a normal karyotype and intellectual development is normal. There is a history of hyperphagia with constant food seeking and distress when food is not provided. Fasting serum leptin concentration is appropriate for the degree of obesity (30 ng/ml). The proband's mother is not obese and has a normal appetite. The proband's father is aged 30 years, his weight is 139 kg, height is 185 cm and BMI 41 kg/m². He was of normal birth weight, but his weight began

to deviate from predicted centiles at six months of age (Fig. 2b). At age six years, he was hospitalized for calorie restriction. He was found to be heterozygous for the deletion at codon 211 of *MC4R*. He has no siblings and no further information concerning his parents is available.

The finding of a phenotype associated with a frameshift mutation in a G-protein coupled receptor gene is suggestive of haplo-insufficiency rather than dominant-negativity as, in general, members of this family of receptors function as monomers⁸. Although this family is insufficiently large to determine the statistical probability of the causal nature of this mutation, the fact that haplo-insufficiency for *Mc4r* in mice leads to obesity provides support for the notion that the *MC4R* mutation and the obesity of the two affected subjects are causally linked.

Thus, *MC4R* represents the first locus at which mutations are associated with dominantly-inherited morbid obesity in man.

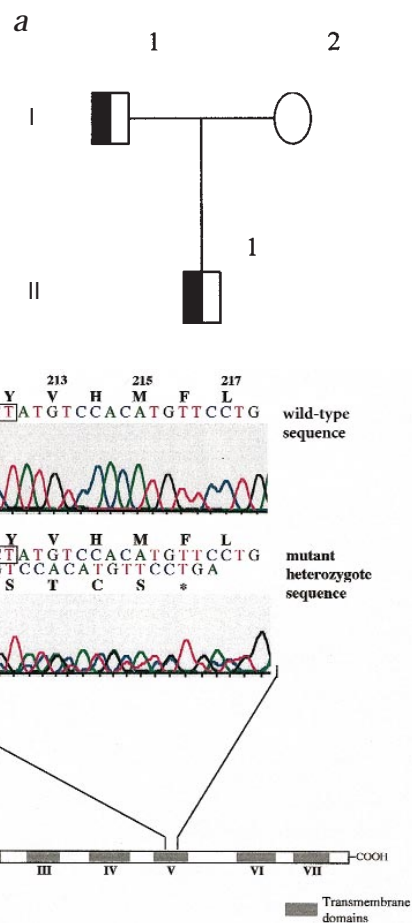
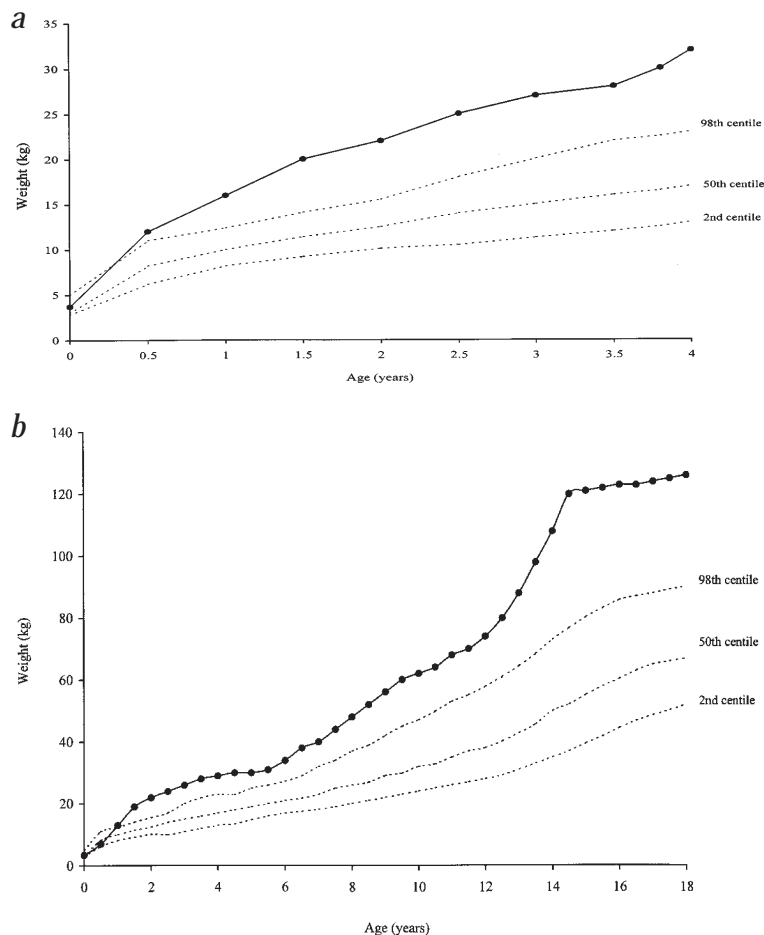


Fig. 1 A frameshift mutation in *MC4R* is associated with dominantly inherited obesity. **a**, Half-filled symbols indicate the heterozygote state of the two obese subjects in this pedigree. **b**, *MC4R* is a 333-aa protein encoded by a single exon of 999 nt. Two primers, *MC4R*forward (5'-AATAACTGAGACGACTCCCTGAC-3') and *MC4R*reverse (5'-CAGAAGTACAATATTCAGGTAGGG-3'), were used in a PCR reaction to amplify the gene from genomic DNA isolated from whole blood. The PCR was performed using BioTaq (Bioline) and carried out under standard conditions, with 35 cycles of 95 °C for 30 s, 57 °C for 30 s and 72 °C for 50 s. Six nested primers, *MC4F1* (5'-TGAGACGACTCCCTGACCCAG-3'), *MC4F2* (5'-CATCACCTATTAACAGTACAG-3'), *MC4F3* (5'-AGGCTTCACATTAAGAGGATTG-3'), *MC4R1* (5'-TACAATATTCAGGTAGGGTAAGA-3'), *MC4R2* (5'-TTGGCGGATGGCACCAGTGC-3') and *MC4R3* (5'-CACTGTGAACTCTGTGCATC-3') were then used, at an annealing temperature of 57 °C, to sequence the resulting PCR product on both strands. Sequencing was carried out using BigDye terminator chemistry (Perkin-Elmer) and electrophoresed on an ABI 377 automated DNA sequencer. The two subjects were heterozygous for a 4-nt 'CTCT' deletion (highlighted with a box) at codon 211, resulting in a missing leucine and a frameshift that introduces a stop codon 5-aa downstream of the mutation. This disrupts the fifth transmembrane domain of the receptor and results in a truncated protein of 215 residues. The amino acid translation for the respective alleles is denoted in bold single letter code with the wild-type residues numbered in arabic numerals and the asterisk denotes a stop codon. The shaded bars in the schematic diagram of *MC4R* below represent each of the seven transmembrane domains and are denoted in roman numerals.

Fig. 2 Auxology for subjects heterozygous for a frameshift mutation in *MC4R*. **a**, Birthweight of subject II.1 was 3.8 kg (50th centile); by four months of age his weight began to deviate from predicted centiles for males. **b**, Birthweight of subject I.1 was 3.5 kg (50th centile); his weight began to deviate from predicted centiles for males by six months of age. He continued to gain weight throughout childhood and at 18 years of age weighed 126 kg.



Homozygous or compound heterozygous mutations in genes encoding leptin^{9,10}, leptin receptor¹¹, prohormone convertase 1 (PC1; ref. 7) and pro-opiomelanocortin (POMC; ref. 6) have been described in association with human obesity; in no instance have heterozygotes been reported to be morbidly obese.

Among the monogenic defects leading to severe human obesity, a phenotype/genotype correlation is beginning to emerge. Hypogonadotropic hypogonadism is found with mutations in the leptin^{9,10}, leptin receptor¹¹ and *PC1* genes (ref. 7), hypoadrenalism with *POMC* (ref. 6) and *PC1* genes (ref. 7) mutations and short stature with leptin receptor gene mutations¹¹. It is notable that the obese *MC4R* mutant subjects show no evidence of impaired adrenal function and that sexual development and fertility is normal in

subject I.1. Both affected subjects in this pedigree are tall, which is of interest given the increased linear growth exhibited by heterozygote *Mc4r*-deficient mice.

These findings add to the evidence attesting to the importance of melanocortin signalling in the control of appetite and weight in mice and humans. Subtle alterations in *MC4R* receptor function may be important in the regulation of weight control and *MC4R* may be a compelling candidate gene for involvement in more common polygenic forms of obesity.

Acknowledgements

We thank G. Cook for her help in establishing the patient cohort and the many clinicians who have referred patients to us. S.O.R. & I.S.F. are supported by the Wellcome Trust.

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